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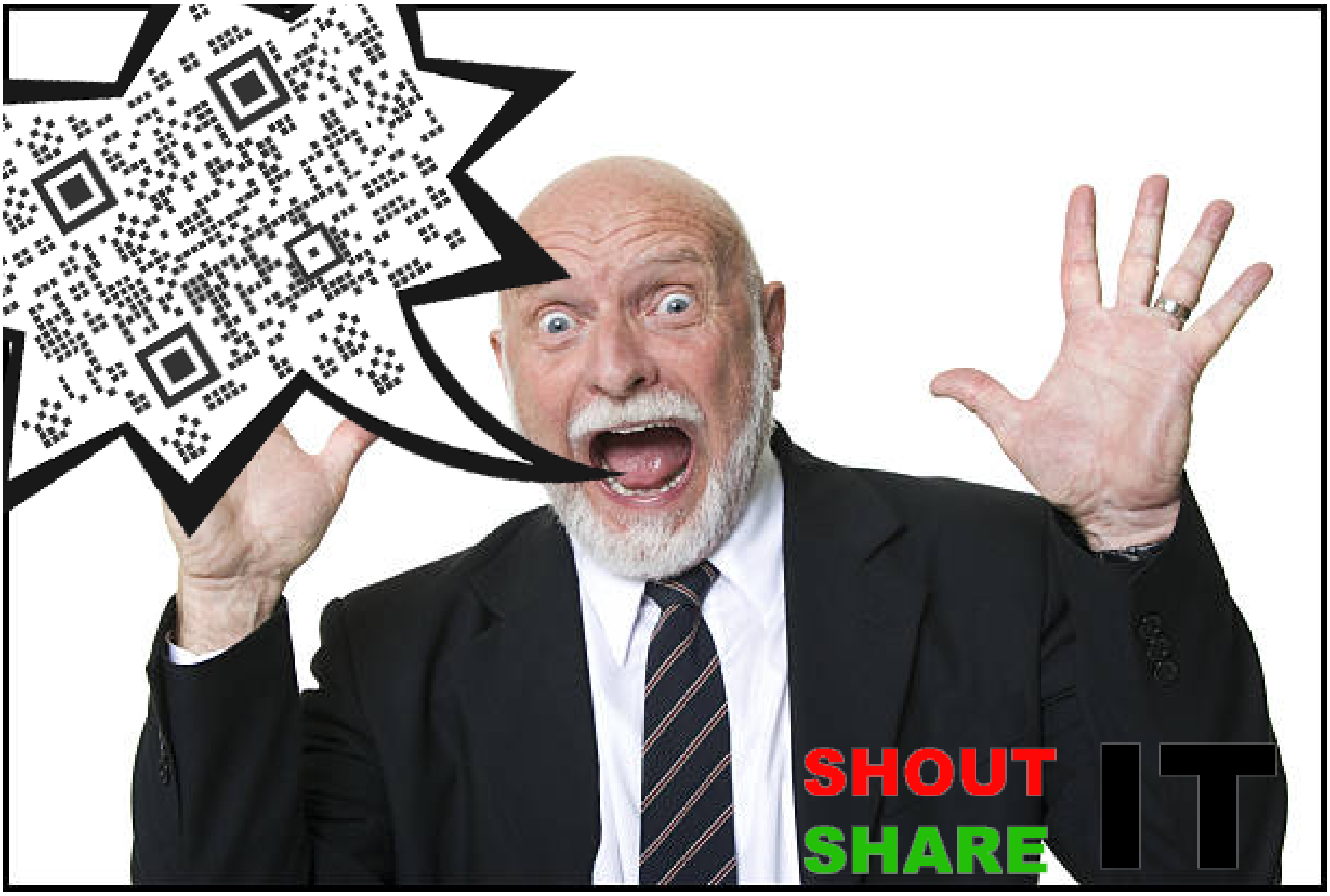
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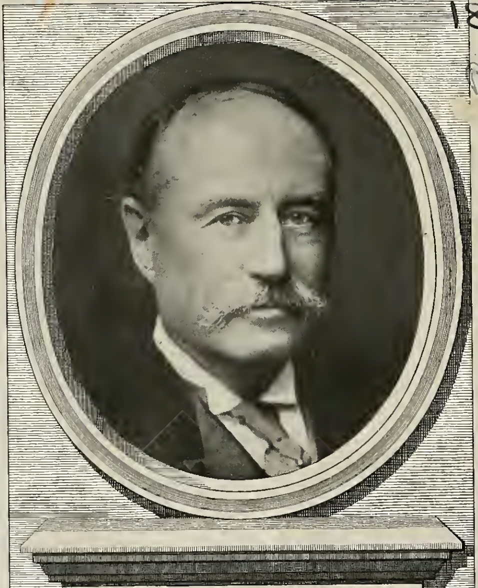

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THE

CHEMICAL BASIS

OF THE

ANIMAL BODY.

[illegible]

THE CHEMICAL BASIS OF THE ANIMAL BODY.

An Appendix to Foster's Text-Book of Physiology.

(SIXTH EDITION.)

BY

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New York:
MACMILLAN AND CO.
AND LONDON.
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PREFACE.

THE following Appendix has been written upon the same lines as in former editions, save that it has been enlarged, and in reality now constitutes a treatise on the chemical substances occurring in the animal body. As in former editions it is entirely the work of Dr. A. Sheridan Lea.

The references given, though extensive, are not intended to be exhaustive. An effort has been made to make the references to recent work as complete as possible; other references are to papers which themselves give full references and will therefore serve as a guide to the literature of the subject; and some have been inserted in order to inform the student of the dates at which important results were first described.

We desire to express our thanks to Messrs. Winter of Heidelberg for the six figures which have been taken from Krukenberg's *Grundriss der medicinisch-chemischen Analyse*, and to Professor Kühne for the large number which have been taken from his *Lehrbuch der physiologischen Chemie* (1868). A few have been drawn in wood from the plates in Funke's *Atlas der physiologischen Chemie* (1858).

We are also indebted to Dr. S. Ruhemann for reading the proofs from page 91 to page 216, in which the text contains many formulæ, and involves special chemical knowledge.

The volume is paged separately from the rest of the Text-Book, and has an index of its own. Indeed it may be regarded as an independent work. The references to the body of the Text-Book are given in paragraphs.

M. FOSTER.
A. SHERIDAN LEA.

July, 1892.

PART V.—APPENDIX.

THE CHEMICAL BASIS OF THE ANIMAL BODY

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APPENDIX.

THE CHEMICAL BASIS OF THE ANIMAL BODY.

THE animal body, from a chemical point of view, may be regarded as a mixture of various representatives of three large classes of chemical substances, viz. proteids, carbohydrates and fats, in association with smaller quantities of various saline and other crystalline bodies. By proteids are meant bodies containing carbon, oxygen, hydrogen, and nitrogen in a certain proportion, varying within narrow limits, and having certain general features; they are frequently spoken of as albuminoids. By carbohydrates are meant starches and sugars and their allies. We have also seen that the animal body may be considered as made up on the one hand of actual 'living substance,' sometimes spoken of as protoplasm (see § 5), in its various modifications, and on the other hand of numerous lifeless products of metabolic activity. We do not at present know anything definite about the molecular composition of the active living substance; but when we submit living substance to chemical analysis, in which act it is killed, we always obtain from it a considerable quantity of the material spoken of as proteid. And many authors go so far as to speak of living substance or protoplasm as being purely proteid in nature; they regard the living protoplasm as proteid material, which in passing from death to life has assumed certain characters and presumably has been changed in construction, but still is proteid matter; they sometimes speak of protoplasm as 'living proteid' or 'living albumin.' It is worthy of notice, however, that even simple forms of living matter, like that constituting the body of a white corpuscle, forms which we may fairly consider as the nearest approach to native protoplasm, when they can be obtained in sufficient quantity for chemical analysis, are found to contain some representatives of carbohydrates and fats as well as of proteids. We might perhaps even go as far as to say that, in all forms of living substance, the proteid basis is found upon analysis to have some carbohydrate and some kind of fat associated with it. Further, not only does the normal food which is eventually built up into living substance consist of all three classes, but, as we have seen in the sections on nutrition, gives rise by metabolism to members of the same three classes; and as far as we know at present, carbohydrates and fats, when formed in the body out of proteid food, are so formed by the agency of living substance, by the action of some living tissue. Hence there is at least some reason for thinking it probable that the molecule of living substance, if we may use such a phrase, is far more complex than a molecule of proteid matter, that it contains in itself residues so to speak not only of proteid, but also of carbohydrate and fatty material.

The plasmodium of Æthalium septicum, a myxomycætous fungus, presents a convenient source of extremely primitive protoplasm which may be obtained in large quantities. It occurs as an extended, yellow, gelatinous mass, frequently of considerable thickness, on the surface of heaps of spent tan or other similar decaying vegetable matter, and exhibits very active movements both internally and more particularly at its edges, of an essentially amœboid nature. It has been carefully analysed by Reinke, *Studien über das Protoplasma*, Berlin, 1881. See also Krukenberg, *Unters. a. d. physiol. Inst. Heidelb.*, Bd. II. 1882, S. 273.

Whether this be so or not, for at present no dogmatic statement can be made, there is no doubt that when we examine the various tissues and fluids of the animal body from a chemical point of view we find present in different places, or at different times in the same tissue or fluid, several varieties and derivatives of the three chief classes; we find many forms of proteids, and bodies closely allied to proteids, in the forms of mucin, gelatine, &c.; many varieties of fats; and several kinds of carbohydrates.

We find, moreover, many other substances which we may regard as stages in the constructive or destructive metabolism of the various forms and phases of living matter, and which are important not so much from the quantity in which they occur in the animal body at any one time as from their throwing light on the nature of animal metabolism; these are such substances as urea, uric acid, other organic crystalline bodies, and the extractives in general.

In the following pages the chemical features of the more important of these various substances which are known to occur in the animal body will be briefly considered, such characters only being described as possess or promise to possess physiological interest. The physiological function of any substance must depend ultimately on its molecular (including its chemical) nature; and though at present our chemical knowledge of the constituents of an animal body gives us but little insight into their physiological properties, it cannot be doubted that such chemical information as is attainable is a necessary preliminary to all physiological study.

PROTEIDS.[1]

These form the principal solids of the muscular, nervous, and glandular tissues, of the serum of blood, of serous fluids, and of lymph. In a healthy condition, sweat, tears, bile, and urine contain mere traces, if any, of proteids. Their general percentage composition may be taken as lying within the following limits:—

C	50·0 to 55·0	50·0 to 55·0
H	6·9 „ 7·3	6·8 „ 7·3
N	15·0 „ 18·0	15·4 „ 18·2
O	20·0 „ 23·5	22·8 „ 24·1
S	0·3 „ 2·0	0·4 „ 5·0
(Hoppe-Seyler.[2])		(Drechsel.)

The composition of the true proteids lies so constantly within the above limits that conclusions as to the proteid nature of any substance whose purity is assured may be drawn with safety from the results of its ultimate analysis. This is important in cases where a substance is with difficulty, if at all, obtained in a condition such that it yields none of the reactions characteristic of proteids. Kühne and Chittenden's analyses[3] of peptones freed from albumoses, which they quote with considerable reserve, alone show a percentage composition lying appreciably outside the above limits.

In addition to the above constituents, proteids ordinarily leave on ignition a variable quantity of ash. In the case of egg-albumin the principal constituents of the ash are chlorides of sodium and potassium, the latter exceeding the former in amount. The remainder consists of sodium and potassium, in combination with phosphoric, sulphuric, and carbonic acids, and very small quantities of calcium, magnesium, and iron, in union with the same acids. There may be also a trace of silica.[4] The ash of serum-albumin contains an excess of sodium chloride, but the ash of the proteids of muscle contains an excess of potash salts and phosphates. The nature of the connection of the ash with the proteid is still a matter of obscurity, and it is not known whether they constitute an integral part of its molecule or are merely adherent impurities. There is a certain amount of probability that the latter is the case, inasmuch as an increasing number of proteids have in recent times been obtained practically free from any ash-residue on ignition. It is, however, possible that in their natural condition as constituents of the animal tissues and fluids the proteids are combined with salts, the separation of which we are now speaking being an artificial result of the processes employed to effect that separation. The sulphur in proteids is present partly in a stably combined condition, partly loosely combined. The latter is removed by boiling with alkalis, the former is not. The proportions of the two differ in the several proteids.[1]

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[1] The chemistry of proteids and allied substances, together with a compendious literature of the subject, is very fully treated and recorded in Drechsel's article "Eiweisskörper" in Ladenburg's *Handwörterbuch der Chemie*, Bd. iii. (1885), S. 534, and in Beilstein's *Handbuch der organischen Chemie*, Bd. iii. (1882–90), S. 1258.

[2] *Hdbch. d. phys. path. chem. Anal.* Auf. 5 (1883), S. 258.

[3] *Zt. f Biol.* Bd. xxii. (1886), S. 452.

[4] Gmelin, *Hdbch. d. org. Chem.* Bd. viii., S. 285.

Proteids met with in the animal body are all amorphous, the only apparent exception being haemoglobin: this substance is however not a pure proteid but a compound of a proteid globin with the less complex haematin. It is to the latter that the power of crystallising is due.

Some are soluble, some insoluble in water, some are characteristically soluble in moderately concentrated solutions of neutral salts, and all are for the most part insoluble in alcohol and ether; they are all soluble in strong acids and alkalis, but in becoming dissolved mostly undergo decomposition. Their solutions exert a left-handed rotatory action on the plane of polarisation, the amount depending on various circumstances, and differing for the several proteids.

Crystals into whose composition certain proteid (globulin) elements largely entered were long since observed in the aleurone-grains of many seeds.[2] Similar crystalloid compounds are also described as occurring occasionally in the egg-yolk of some animals (Amphibia and Fishes). By appropriate methods they may be separated and recrystallized from their solution in distilled water, most readily by Drechsel's method of alcohol dialysis.[3] The crystals consist in no case of pure proteids, but are always compounds of the latter with some inorganic residue such as lime or magnesia. These recrystallized, and hence presumably pure, compounds have been frequently analysed with a view to establishing a formula for proteids which should give some clue to their molecular magnitude. An excellent summary of the endeavours to arrive at a definite formula for proteids, based on the above analyses and on those of haemoglobin and certain compounds of egg-albumin with salts of copper and silver is given by

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[1] A. Krüger, Pflüger's *Arch.* Bd. xliii. (1888), S. 244.

[2] For literature down to the year 1877, see Weyl, *Zt. f. physiol. Ch.* Bd. i., S. 84. See also Hoppe-Seyler's *Handbuch*, Ed. v. p. 259. Vines, *Jl. of Physiol.* Vol. iii. (1880), p. 102. Chittenden and Hartwell, *Jl. of Physiol.* Vol. xi. (1890), p. 435.

[3] *Jl. f. prakt. Chem.* N. F. Bd. xix. (1879), S. 331.

Bunge.[1] As the result of these, various formulae have been proposed by the several observers. Very little real importance can however be attached to these formulae, for, as Drechsel observes, in so large a molecule an analytical error of ·01 p.c. would have the same importance as would one of ·1 p.c. in ordinary analyses. They give us at most an idea of the *minimal* magnitude of the proteid molecule, but apart from this they throw no more light on the subject than already existed in Lieberkühn's older formula.

General reactions of the proteids.[2]

1. Heated with strong nitric acid, they or their solutions turn yellow, and this colour is, on the addition of ammonia, or caustic soda or potash, changed to a deep orange hue. (Xanthoproteic reaction.)

If much proteid, except albumoses and peptones, be present a yellow precipitate is obtained at the same time. With less proteid their solutions merely turn yellow on boiling and orange on the addition of the alkali: if only a *trace* of proteid is present no yellow colour is observed until after the addition of the alkali.

2. With Millon's reagent[3] they give, when present in sufficient quantity, a precipitate, which turns red on heating. If they are only present in traces, no precipitate is obtained, but merely a red colouration of the solution when heated.

3. If mixed with an excess of concentrated solution of sodium hydrate, and one or two drops of a dilute solution of cupric sulphate, a violet colour is obtained, which deepens in tint on boiling. (Piotrowski's reaction.[4])

The above serve to detect the smallest traces of all proteids.

4. Render the fluid strongly acid with acetic acid, and add a few drops of a solution of ferrocyanide of potassium; a precipitate shews the presence of proteids, except true peptones and some forms of albumose.

5. Render the fluid, as before, strongly acid with acetic acid, add an equal volume of a concentrated solution of sodium sulphate, and boil. A precipitate is formed if proteids, except peptones, are present.

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[1] *Lehrb. d. physiol. u. path. Chem.* 1887, Sn. 52–58. For most recent analysis of haemoglobin from dog's blood see Jaquet, *Zt. f. physiol. Ch.* Bd. XII. (1888), S. 285, XIV. S. 289. Chittenden and Whitehouse, *Stud. Lab. physiol. Chem. Yale*, Vol. II. (1887), p. 95.

[2] Consult in all cases Hoppe-Seyler's *Hdbch. d. physiol. path. chem. Analyse.* Ed. V. 1883. See also Krukenberg, *Sitzb. d. Jena. Gesell. f. Med. u. Natwiss.* 1885, Nr. 2.

[3] *Compt. Rend.* T. XXVIII. (1849), p. 40.

[4] *Sitzb. d. Wien. Akad.* Bd. XXIV. (1857), S. 335.

This reaction is particularly useful, not merely because it effects a very complete precipitation of the proteids which are present (except peptones), but also because the reagents employed do not produce any decomposition of other substances which may be present, and do not interfere with certain other tests which it may be necessary to apply after the removal of the proteids by filtration. It is of use more particularly in the determination of sugar in blood.[1]

The following reactions are specially used for freeing solutions from all proteids by precipitation.

6. Acidulate *faintly* with acetic acid and add tannic acid.

7. Acidulate with hydrochloric acid and add the double iodide of mercury and potassium. (Brücke's reagent.[2])

8. Add hydrochloric acid until the reaction is *strongly* acid; then add phosphotungstic acid.

The following methods are often additionally useful for freeing solutions from all proteids.

i. Precipitate by excess of absolute alcohol, having previously made the solution neutral or faintly acid.

ii. Prepare a solution of ferric acetate by *saturating* acetic acid with freshly precipitated ferric oxide, avoiding all excess of free acid. Add this to the solution and boil; the whole of the proteids are precipitated together with the iron; the latter as a basic salt.[3] In some cases a mixture of ferric chloride and an *excess* of sodium acetate is employed.[4]

iii. Boil the solution for a few minutes with a little hydrated oxide of lead in presence of a little lead acetate.[5]

In recent years various neutral salts, more particularly neutral ammonium sulphate,[6] have been largely employed for effecting the precipitation and separation of the several proteids.

All proteids yield a characteristic violet colouration with simultaneous slight fluorescence upon treatment with glacial acetic acid and strong sulphuric acid (Adamkiewicz' reaction). The reaction is best obtained by adding to the suspected solution or substance a mixture of one volume of strong sulphuric acid and two volumes of glacial acetic acid and boiling.[7] The violet-coloured solution observed if proteids are present gives an absorption band between the lines *b* and *F* in the solar spectrum.

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[1] See Gamgee's *Physiol. Chem.* Vol. I. p. 195.

[2] *Sitzb. d. Wien. Akad.* LXIII. 2 (1871), Feb. Hft.

[3] Hoppe-Seyler, *Hdbch.* S. 264.

[4] Seegen, Pflüger's *Arch.* Bd. XXXIV. (1884), S. 391.

[5] Hofmeister, *Zt. f. physiol. Chem.* Bd. II. (1878), S. 288.

[6] Wenz, *Zt. f. Biol.* Bd. XXII. (1886), S. 10. Kühne, *Verhand. d. Naturhist.-Med. Ver. Heidelb.* N. F. Bd. III. 1885, S. 286. See also Halliburton, *Jl. of Physiol.* Vol. V. (1883), p. 172.

[7] Hammarsten, Pflüger's *Arch.* Bd. XXXVI. (1885), S. 389.

No general method can be given for the quantitative estimation of the various proteids. For this some special manuals should be consulted and use made of the reactions which are specifically characteristic of each proteid as given below.

Solutions of different proteids rise to different heights in capillary tubes. It is possible that this fact may be of use in detecting and estimating their approximate relative amounts.[1]

Classification of the Proteids.[2]

The following classification is both convenient and concise.

Class I. *Native albumins.*

Soluble in distilled water. Solutions coagulated on heating, especially in presence of a *dilute* (acetic) acid. Not precipitated by carbonates of the alkalis or by sodium chloride, or generally by solutions of neutral salts.

1. Egg-albumin. Serum-albumins.

Class II. *Derived albumins* (*Albuminates*).

Insoluble in distilled water and in dilute neutral saline solutions; soluble in acids and alkalis. Solutions not coagulated by boiling.

1. Acid-albumin. 2. Syntonin. 3. Alkali-albumin. 4. Casein or Native alkali-albumin.[3]

Class III. *Globulins.*

Insoluble in distilled water, soluble in dilute saline solutions. Soluble in *very dilute* acids and alkalis: if the acids and alkalis are strong they are rapidly changed into members of Class II. Readily precipitated by saturating their dilute saline solutions with neutral salts such as sodium chloride or magnesium sulphate.

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[1] Bodländer u. Traube, *Ber. d. deutsch. chem. Gesell.* Bd. XIX. (1886), S. 1871.

[2] See Hoppe-Seyler, *Hdbch* Ed. v. S. 265. Drechsel in Ladenburg's *Handwörterbuch d. Chem.* Bd. III. S. 550. Danilewski, *Arch. d. Sci. phys. et nat.* (3) T. 7 (1882), Nr. 4.

[3] Casein differs in many respects from the other members of this class, but in its general reactions is more closely allied to them than to the members of any other class. In its ready precipitability by neutral salts it shews some affinity to the globulins.

1. Crystallin, the globulin of the crystalline lens. 2. Vitellin. 3. Paraglobulin or Serum-globulin. 4. Fibrinogen. 5. Myosin. 6. Globin.

Class IV. *Fibrins.*

Insoluble in water. Soluble with difficulty in *strong* acids and alkalis, and undergoing a simultaneous change into members of Class II. Soluble by the prolonged action of moderately strong (10 p.c.) solutions of neutral salts, with simultaneous change into members of Class III.

Class V. *Coagulated proteids.*

Products of the action of heat on members of Classes I., III., and IV., or of Class II. when precipitated by neutralisation and heated in suspension in water. They are also obtained by the prolonged action of alcohol in excess upon members of Classes I., III., and IV. Their solubilities, except in solutions of neutral salts, are in general similar to, but less than those of Class IV.

Class VI. *Albumoses and peptones.*[1]

The true peptones are extremely soluble in water. They are not precipitated by acids, alkalis, neutral salts, or many of the reagents which precipitate other proteids. They are precipitated but not coagulated by even the prolonged action of alcohol. Peptones are readily diffusible, albumoses less so. Some of the albumoses are readily soluble in water, some are less soluble. They are distinguished from peptones by being precipitated when their solutions are saturated with neutral ammonium sulphate. They yield precipitates with many of the reagents which precipitate other proteids, and it is specially characteristic that the precipitates they yield with nitric acid and with ferrocyanide of potassium in presence of acetic acid disappear when warmed and reappear on cooling.

Class VII. *Lardacein or amyloid substance.*

Insoluble in water, dilute acids and alkalis, and saline solutions. Converted into members of Class II by strong acids and alkalis.

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[1] The albumoses are classed with the peptones partly from their close relationship to these substances and partly for convenience.

The Chemistry of the several Proteids.[1]

Class I. *Native Albumins.*

1. **Egg-albumin.**

As obtained in the solid form by evaporating its solutions to dryness at 40°, preferably in vacuo, it forms a semi-transparent, brittle mass, of a pale yellow colour, tasteless and inodorous. Dissolved in water it yields a clear neutral colourless solution. This solution coagulates on heating, but the temperature at which the coagulation takes place varies considerably with the concentration, and is largely dependent upon the presence or absence of salts. The more commonly observed temperature is 70–73°, but Gautier states[2] that coagula may also be obtained at 54° and 63°. The more dilute the solution is, the higher is the temperature at which it coagulates, thus finally resembling a solution of albumin from which the salts have been removed by dialysis.[3] When precipitated from solution by excess of alcohol it is readily coagulated by the precipitant, so that it is now usually insoluble in water. In this respect it differs somewhat characteristically from serum-albumin, which is not so immediately, though it is ultimately, coagulated by the action of alcohol.

According to Corin and Berard,[4] by applying the method of fractional heat-coagulation to filtered white of egg, coagula may be obtained at 57·5°, 67°, 72°, 76°, and 82°, the first two being due to globulins, the others to albumins.

Strong acids, especially nitric acid, cause a coagulation similar to that produced by heat or by the prolonged action of alcohol; the albumin becomes profoundly changed by the action of the acid, and does not dissolve upon removal of the acid. Mercuric chloride, nitrate of silver, and lead acetate, precipitate the albumin, forming with it insoluble compounds of variable composition.

Strong acetic acid in excess gives no precipitate, but when the solution is concentrated the albumin is transformed into a transparent jelly. A similar jelly is produced when strong caustic potash is added to a concentrated solution of egg-albumin. In both these cases the substance is profoundly altered, becoming in the one case acid- in the other alkali-albumin.

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[1] In addition to the works already quoted consult Beilstein, *Hdbch. d. org. Chem.* Bd. III. (1889), Sn. 1258–1310, for all data concerning the proteids.

[2] *Chimie appliquée à la Physiol. &c.* T. I. (1874), p. 242. Haycraft and Duggan, *Proc. Roy. Soc. Edinb.* 1889, p. 364. Starke (Swedish). See Abst in Maly's *Jahresbericht*, XI. (1881), S. 19.

[3] Laptschinsky, *Sitzb. d. Wien. Akad.* Bd. LXXVI. 1877. Juli-Hft.

[4] *Travaux du Lab. de Léon Frédéricq, Liége.* T. II. (1888), p. 170.

The specific rotatory power, which is stated to be independent of the concentration, is variously given as $(\alpha)_D = -35{\cdot}5°$ (Hoppe-Seyler), or $-37{\cdot}79°$ (Starke). The latter agrees closely with Haas' determination[1] $(\alpha)_D = -38{\cdot}1°$ and is probably the most correct of the three values.

Preparations. The fibrous network in white of egg is broken up with scissors and violently agitated in a flask till a thick froth is formed. The flask is then inverted, whereupon the foam rises to the top, carrying the larger part of the fibrous *débris* with it. The clear subnatant fluid is now carefully drawn off and filtered through fine muslin; to this an equal volume of water is added, and the whole is finally filtered through coarse paper. From this point onwards two methods may be employed.

1. For ordinary purposes the fluid may be very carefully and faintly acidulated with acetic acid, filtered and the filtrate purified by dialysis.

2. To obtain the purest albumin proceed as follows:[2] Saturate the fluid with magnesium sulphate at 20°, filter and saturate the filtrate with sodium sulphate. Dissolve the precipitate of albumin thus obtained in water and precipitate again with the sodium salt, and after repeating this process several times remove the last traces of salt by dialysis and concentrate to dryness at 40°.

According to recent researches egg-albumin may be obtained in a crystalline form by slow evaporation of its solutions in presence of neutral ammonium sulphate. The separation takes place at first in the form of minute spheroidal globules of various sizes, and finally minute needles, either aggregated or separate, make their appearance. It has not as yet been found possible to obtain these so-called crystals from solutions which have been freed by dialysis from the ammonium salt. Further investigation is needed to establish their real nature.[3]

The primary digestive products obtained during the peptic digestion of egg-albumin have been studied by Chittenden and Bolton.[4]

2. **Serum-albumin.**

This is the sole proteid, apart from the globulins, which occurs in serum.[5] Pure solutions of this proteid closely resemble those of egg-albumin in their general reactions, but the difference of the two is clearly shewn by the following statements:—

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[1] Pflüger's *Arch.* Bd. XII. (1876), S. 378.

[2] Starke, *loc. cit.*

[3] Hofmeister, *Zt. f. physiol. Chem.* Bd. XIV. (1889) S. 165. Gabriel, *ibid.* Bd. XV. Hf. 5 (1891) S. 456.

[4] *Stud. Lab. Physiol. Chem. Yale Univ.* Vol. II. (1887), p. 126.

[5] 'Serum casein' of Kühne and Eichwald was shewn by Hammarsten to consist

1. When free from salts and in 1—1·5 p.c. solution it coagulates on heating to 50°. The addition of sodium chloride raises the coagulating point to 75°—80°.[1] Under the conditions in which it occurs in serum it is not found to shew any opalescence on heating at any temperature below 60°, and it may be regarded as coagulating completely at 75.°

By fractional heat-coagulation of serum freed from globulin Halliburton [2] has obtained evidence of the existence in the serum of many animals of three albumins coagulating at 70–73°, 77–78°, and 82–85°. In some serum only two of these albumins occur.

2. It is not readily coagulated by alcohol or precipitated by ether: egg-albumin is, and most readily by alcohol.

3. It is difficult to make any one definite statement as to the specific rotatory power of serum-albumin since it appears to differ for the substance as obtained from different animals. Starke gives it as $(\alpha)_{\mathrm{D}} = -62{\cdot}6^\circ$ for human serum-albumin, and $-60{\cdot}05^\circ$ for that of the horse.

4. It is not very readily precipitated by strong hydrochloric acid, and the precipitate is readily soluble on the further addition of acid: the reverse is the case for egg-albumin.

5. Precipitated or coagulated serum-albumin is more readily soluble in nitric acid than is egg-albumin.

6. When precipitated by alcohol it is, as already stated, less immediately though it is ultimately coagulated by the action of the precipitant, than is egg-albumin.

7. According to Gauthier[3] the following reagent precipitates egg-albumin but not serum-albumin: 250 c.c. caustic soda, sp. gr. 0·7: 50 c.c. sulphate of copper 1 p.c.: 700 c.c. glacial acetic acid. To be added in the ratio of 10 c.c. to 2 c.c. of the fluid to be tested.

8. Egg-albumin if injected subcutaneously or into a vein, reappears unaltered in the urine; serum-albumin similarly injected does not thus normally pass out by the kidney.

Serum-albumin is found not only in blood-serum, but also in lymph, both that contained in the proper lymphatic channels and

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really of serum-globulin, and this is confirmed by Halliburton, *Jl. Physiol.* Vol. v. (1883), p. 193.

[1] Starke, *loc. cit.* S. 18.

[2] *Jl. Physiol.* Vol. v. 1883, p. 152. But see also Vol. xi. (1890), p 453.

[3] See Maly's *Ber.* Bd. xv. (1885), S. 31.

that diffused in the tissues; in chyle, milk, transudations, and many pathological fluids.

It is this form in which albumin generally appears in the urine.

Scherer described two proteids which he obtained from the contents of ovarial cysts, and to which he gave the names of metalbumin and paralbumin.[1] Hammarsten concludes from his researches[2] that they are really identical. Metalbumin seems to be associated with some carbohydrate substance resembling glycogen (?), since it yields, on heating with sulphuric acid, a body which reduces Fehling's fluid as does dextrose.[3]

Neither egg- nor serum-albumin can be obtained in a condition such that they leave no ash residue on ignition. Al. Schmidt asserted[4] that they could be by means of dialysis, and that in this condition they were no longer coagulable by heat. On this point a keen controversy was carried on for some time, for the details of which see Rollett's article on Blood in Hermann's *Hdbch. d. Physiol.* Bd. IV. Th. 1, S. 93. The whole difficulty seems to have turned on the extreme sensitiveness of dialysed solutions of albumin to the presence or absence of *traces* of acid or alkali, and on the fact that such dialysed albumin is largely changed into an albuminate.[5]

Preparations of pure serum-albumin. Centrifugalised serum is saturated at 30° with magnesium sulphate, and the precipitated globulin[6] is washed on the filter with a saturated solution of the salt. The filtrates are then saturated at 40° with sodium sulphate; by this means the serum-albumin is precipitated. The precipitate is dissolved in water, reprecipitated by sodium sulphate, and the process repeated several times. The final product is then freed from salts by dialysis, precipitated by excess of alcohol, washed with this, and finally with ether, and dried by exposure to the air.[7]

The facts on which this method is based were clearly stated by Denis.[8] Schäfer rediscovered[9] the precipitability of serum-albumin by sodium sulphate in presence of the magnesium salt. Halliburton has shewn[10] that this is due to the action of the double sulphate of magnesium and sodium $MgNa_2(SO_4)_2\ 6\ H_2O$.

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[1] *Ann. d. Chem. u. Pharm.* Bd. 82 (1852), S. 135.

[2] Maly's *Ber.* Bd. XI. (1881), S. 11. *Zt. physiol. Ch.* Bd. VI. (1882), S. 194.

[3] Landwehr, Pflüger's *Arch.* Bd. XXXIX. (1886), S. 203. *Zt. physiol. Chem.* Bd. VIII. (1883), S. 114. Hilger, *Annal. d. Chem.* Bd. 160 (1871), S. 338. Plósz, Hoppe-Seyler's *Med.-Chem. Unters.* (1871), S. 517. Obolensky, Pflüger's *Arch.* Bd. IV. (1871), S. 346.

[4] Pflüger's *Arch.* XI. (1875), S. 1.

[5] Werigo, Pflüger's *Arch.* Bd. XLVIII. (1890), S. 127.

[6] Hammarsten, *Zt. f. physiol. Ch.* Bd. VIII. (1884), S. 467.

[7] Starke, *loc. cit.* (sub. egg-albumin), S. 18.

[8] *Etudes sur le sang*, Paris, 1859, p. 39.

[9] *Jl. of Physiol.* Vol. III. (1880), p. 184.

[10] *Ibid.* Vol. V. 1883, p. 181.

CLASS II. *Derived Albumins* (*Albuminates*).

1. **Acid-albumin.**

When a native albumin in solution, such as egg- or serum-albumin, is treated for some little time with a dilute acid, such as hydrochloric, its properties become entirely changed. The most marked changes are (1) that the solution is no longer coagulated by heat; (2) that when the solution is carefully neutralised the whole of the proteid is thrown down as a precipitate; in other words, the serum-albumin, which was soluble in water, or at least in a neutral fluid containing only a small quantity of neutral salts, has become converted into a substance insoluble in water or in similar neutral fluids. The body into which serum-albumin thus becomes converted by the action of an acid is spoken of as *acid-albumin*. Its characteristic features are that it is insoluble in distilled water, and in neutral saline solutions, such as those of sodic chloride, that it is readily soluble in dilute acids or dilute alkalis, and that its solutions in acids or alkalis are not coagulated by boiling. When suspended, in the undissolved state, in water, and heated to 75° C., it becomes coagulated, and is then undistinguishable from coagulated serum-albumin, or indeed from any other form of coagulated proteid. It is evident that the substance when in solution in a dilute acid is in a different condition from that in which it is when precipitated by neutralisation. If a quantity of serum- or egg-albumin be treated with dilute hydrochloric acid, it will be found that the conversion of the native albumin into acid-albumin is gradual; a specimen heated to 75° C. immediately after the addition of the dilute acid, will coagulate almost as usual; and another specimen taken at the same time will give hardly any precipitate on neutralisation. Some time later, the interval depending on the proportion of the acid to the albumin, on temperature, and on other circumstances, the coagulation will be less, and the neutralisation precipitate will be considerable. Still later the coagulation will be absent, and the whole of the proteid will be thrown down on neutralisation.

The conversion of the native albumins in solution into acid-albumin by dilute acids is facilitated by heating to temperatures below those at which the albumins respectively coagulate.[1] The conversion is extremely rapid if a strong acid is added to a concentrated solution of the proteid; thus when a little glacial acetic acid is stirred into undiluted white of egg the whole solidifies into a yellow transparent jelly consisting of acid-albumin. A similar jelly is formed, only gradually, if the albumin is placed in a ring-dialyser and floated on dilute acids (1—2 p. c.)[1]

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[1] Rollett, *Sitzb. d. Wien. Akad.* Bd. LXXXIV. (1881), S. 332. Heynsius, Pflüger's *Arch.* Bd. XI. (1875), S. 624.

Globulins are more readily converted into acid-albumin than are the native albumins. Coagulated proteids or fibrin require for their conversion the application of the acids, preferably hydrochloric, in a concentrated form, the products thus obtained being practically indistinguishable from the products of the action of dilute acids on the more readily convertible proteids. As obtained by the action of acids on the various proteids the products exhibit certain not very marked differences, which however indicate that each proteid yields its own special acid-albumin. The researches of Mörner[2] have shewn that, contrary to earlier views,[3] acid-albumins differ distinctly from the alkali-albumins. These differences may be more appropriately considered after the preparation and properties of the latter have been described.

Preparation 1. Serum or diluted white of egg is digested at 40—50° for several hours with 1—2 p.c. hydrochloric acid. The solution is now filtered, carefully neutralised, the precipitate collected on a filter and washed with distilled water.

2. Acid-albumin may be rapidly prepared by adding glacial acetic acid to white of egg which has been chopped with scissors and strained through muslin. A jelly is thus formed which can be dissolved in warm water, and from this solution the acid-albumin can be precipitated by neutralisation and washed as before.

2. Syntonin.

Although this substance is merely the acid-albumin which results from the action of acids on the globulin (myosin) contained in muscles, and in its more obvious properties is at first sight identical with other acid-albumins, it merits a short and separate description, not only on account of its historical interest in the chemistry of muscles, but also because recent work has shewn it to be distinctly different from the similar products of the action of acids on other proteids, and its properties and reactions have been more fully studied than those of any other form of acid-albumin.

Liebig, unacquainted with the existence of myosin in the dead muscle, was the first to prepare it by the action of dilute (·1 p.c.) hydrochloric acid on the muscle substance,[4] and he regarded it as the chief and characteristic proteid of muscles (muscle-fibrin). Kühne, however, shewed in his famous researches on muscle-plasma[1] that its formation is due to the conversive action of the acid on myosin.

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[1] Johnson, *Jl. Chem. Soc.* 1874, p. 734. *Ber. d. deutsch. chem. Gesell.* 1874, S. 826. Rollett, *loc. cit.*

[2] The original is in Swedish, but is fully abstracted in Maly's *Jahresbericht*, Bd. VII. (1877), S. 9, and is also published in extenso in Pflüger's *Arch.* Bd. XVII. (1878), S. 468. A convenient *résumé* is given on p. 541.

[3] Soyka, Pflüger's *Arch.* Bd. XII. (1876), S. 347.

[4] *Annalen d. Chem. u. Pharm.* Bd. 73 (1850), S. 125.

Preparation. By the action of 0·1 p.c. hydrochloric acid on pure myosin (see below), or by treatment of finely chopped and *thoroughly washed* muscle substance, preferably from the frog, with the same acid. It may be precipitated from its solution by neutralisation, and freed from salts by washing, but in this case care must be exercised as to the extent of the washing, since syntonin is distinctly altered by the prolonged action of water, especially as regards its solubility in dilute acid and lime-water.[2]

The reactions specially characteristic of this substance and its distinction from other forms of acid-albumin and from alkali-albumin are indicated in the following statements.[3]

1. It is soluble in lime-water, and this solution is coagulated, though incompletely, by boiling (Kühne).

2. It is insoluble in acid phosphate of soda (NaH_2PO_4); other acid-albumins are soluble (Mörner). In presence of this salt it does not pass into solution on the addition of alkali until the whole of the acid phosphate has been converted into the neutral (Na_2HPO_4). In this respect it differs from alkali-albumin, which is soluble under the same conditions long before the conversion of the acid into the neutral phosphate is complete.

3. It is soluble in dilute sodium carbonate.

4. When precipitated from its acid solution by neutralisation the precipitate is more gelatinous than that of the other acid-albumins, and less readily soluble in alkalis (Mörner).

5. Its specific rotatory power when dissolved in dilute hydrochloric acid or sodium carbonate is independent of the concentration, and is given as $(a)_D = -72°$ (Hoppe-Seyler).

Syntonin has been stated to be capable of reconversion into myosin, or some globulin closely resembling it, by solution in lime-water, addition of ammonium chloride to an amount just short of saturation, and neutralisation with acetic acid. The neutral fluid thus finally obtained is allowed to fall drop by drop into distilled water, from which a fine coagulum gradually separates out consisting of myosin.[4] Hoppe-Seyler states that by similar treatment all forms of acid-albumin may be converted into globulins resembling myosin.[5]

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[1] *Ueber das Protoplasma*, Leipzig, 1864, S. 15.
[2] Kühne, *loc. cit.* S. 16. Sander, *Arch. f. Physiol.* Jahrg. 1881, S. 198.
[3] See Mörner, *loc. cit.*
[4] A. Danilewsky, *Zt. f. physiol. Chem.* Bd. v. (1881), S. 158.
[5] *Hdbch. d. chem. Anal.* Ed. v. (1883), S. 281.

3. **Alkali-albumin.**

If serum- or egg-albumin or washed muscle be treated with dilute alkali instead of with dilute acid, the proteid undergoes a change in many ways similar to that which was brought about by the acid. The alkaline solution, when the change has become complete, is no longer coagulated by heat, the proteid is wholly precipitated on neutralisation, and the precipitate, insoluble in water and in neutral solutions of sodium chloride, is readily soluble in dilute acids or alkalis.

Alkali-albumin may be prepared by the action not only of dilute alkalis but also of strong caustic alkalis on native albumins as well as on coagulated albumin and other proteids. The jelly produced by the action of caustic potash on white of egg (p. 11) is alkali-albumin; the similar jelly produced by strong acetic acid is acid-albumin.

In short, the general statement may be made that under otherwise similar conditions, if an alkali is employed instead of an acid to act on proteids, alkali-albumin is formed instead of acid-albumin. In the opinion of many authors[1] the precipitates obtained by neutralising the acid or alkaline solutions which arise during the preparation of acid- and alkali-albumin respectively are to be regarded as identically the same. According to this view the neutralisation precipitate is itself neither acid- nor alkali-albumin, but becomes either the one or the other by solution in either an acid or alkali, entering at the same time into union with the acid or alkali.

Danilewsky[2] has utilised the tropaeolins for the purpose of determining the fixation of acids or alkalis by proteids, and on this he has based a classification of these substances. The tropaeolins are soluble in water, the one (tropaeolin OO) yielding a yellow, the other (tropaeolin OOO No. 1) an orange solution. The first is changed to a lilac colour by acids, but not by salts which have an acid reaction to litmus. The second is turned to bright carmine by free alkalis, but not by salts which have an alkaline reaction to litmus.

It is however on the whole more probable[3] that acid- and alkali-albumin are distinct, though very closely allied substances, and we might go even so far as to say that probably every proteid yields its own kind of either the one or the other proteid on treatment with acids and alkalis. But as yet we do not possess any means of distinguishing between the several forms of each substance by any ordinary reactions.

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[1] Soyka, Pflüger's *Arch.* XII. (1876), S. 347.

[2] *Centralb. f. d. med. Wiss.* 1880, No. 51.

[3] Mörner, Pflüger's *Arch.* Bd. XVII. (1878), S. 468. But see also Kieseritzky, *Inaug.-Diss.*, Dorpat, 1882. Abstr. in Maly's *Jahresber.* Bd. XII. (1882), S. 6, and Rosenberg, *Inaug.-Diss.*, Dorpat, 1883. Abstr. in Maly, Bd. XIII. (1883), S. 19.

The chief though somewhat unsatisfactory evidence which is advanced as to the difference of the two products is the following:

1. Alkali-albumin is in general more soluble than acid-albumin.

2. When precipitated by neutralisation the former (alkali) is flocculent, the latter (acid) is more viscid, transparent, and gelatinous.

3. When dissolved in a minimum of alkali and heated to 100° in sealed tubes, alkali-albumin coagulates, acid-albumin does not.

4. When alkali-albumin is dissolved in Na_2HPO_4 it is not precipitated on the addition of an acid until all the salt has been converted into NaH_2PO_4.[1] (Cf. above, p. 17.)

5. Acid-albumin can be converted into alkali-albumin by the action of strong alkalis, but the reverse conversion of the product thus obtained or of an ordinarily prepared alkali-albumin into acid-albumin is stated to be impossible.

The rotatory power of alkali-albumin varies according to its source; thus when prepared by strong caustic potash from serum-albumin, the rotation rises from $-56°$ (that of serum-albumin) to $-86°$, for yellow light. Similarly prepared from egg-albumin, it rises from $-38{\cdot}5°$ to $-47°$, and if from coagulated white of egg, it rises to $-58{\cdot}8°$. Hence the existence of various forms of alkali-albumin is probable.

The substance 'protein,' described by Mulder,[2] appears, if it exists at all, to be closely connected with this body. All subsequent observers have however failed to confirm his views, and it is only mentioned here from its historical interest. Since Mulder's time the name has been applied to various forms of proteid.

Preparation. The best method is that originally introduced by Lieberkühn.[3] Purified white of egg (see p. 11) is made into a jelly by the addition with rapid stirring of *strong* caustic soda, avoiding as far as possible all excess of the latter. The jelly is then cut into *small* lumps and washed in distilled water, *frequently changed,* until the lumps are quite white throughout. The lumps of purified albumin are then dissolved in water by gently heating on a water-bath, the solution filtered, and the alkali-albumin precipitated by careful addition of acetic acid. The precipitate is then thoroughly washed with distilled water.

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[1] Soyka, *loc. cit.* See also Soxhlet, *Jn. f. prakt. Chem.* N. F. Bd. VI. (1872), S. 1.

[2] *Ann. d. Ch. u. Pharm.* Bd. XXVIII. (1838), S. 81.

[3] Poggendorf's *Annal.* Bd. LXXXVI. S. 118.

The product thus obtained is very pure, but there is a considerable loss of material during the washing of the gelatinous lumps, owing to the solubility of the substance in the alkali which is being removed. The pure substance itself is also slightly soluble in water.

4. **Casein.**[1]

This is the well-known proteid existing characteristically in milk and in no other fluid or secretion of the body.[2]

It has recently been proposed to call this proteid 'caseinogen' and to use the name casein for the product of its decomposition, the clot or curd, which is formed by the action of rennin upon it. This nomenclature would have the advantage of indicating a relationship between the two proteids similar to that between fibrin and fibrinogen, myosin and myosinogen (Halliburton).

Preparation.[3] Fresh milk is diluted with 4 volumes of distilled water and acidulated with acetic acid until the diluted milk contains from ·075 to 0·1 p.c. of the acid. If the milk has been diluted with ordinary tap-water rather more acid must be added. The precipitated casein is now washed two or three times by decantation with water, *as rapidly as possible,* dissolved in the least quantity of dilute caustic soda which suffices for its solution, and filtered through a series of filters until the filtrate is quite clear and only faintly opalescent. This filtrate is then somewhat diluted, the casein again precipitated by the careful addition of acetic acid, and the whole process of washing, solution, and reprecipitation carried out a second time. The final product is now freed as far as possible from water, worked up into an emulsion with 97 p.c. alcohol, collected on a filter, washed with alcohol, finally with ether, dried by exposure to the air, and finally in vacuo over sulphuric acid.

Casein may also be separated from milk by precipitation with an excess of sodium chloride[4] or magnesium sulphate.[5] The latter procedure is chiefly of use for the preparation of casein from human milk, from which it can scarcely be precipitated by means of acids.

Pure casein as obtained by the above method is a fine, snow-white powder, which on ignition of even large quantities of the substance (4—6 grm.) leaves scarcely a trace of ash. It is practically insoluble in water, but is soluble in alkalis, carbonates and phosphates of the alkalis, lime- and baryta-water. From these solutions it may be precipitated by excess of neutral salts such as sodium chloride, and by dilute acids, in which it is again soluble if any excess of acid is present. Its reactions thus correspond closely to those of acid- and alkali-albumin, but as will be presently shewn it is in many ways perfectly distinct from these substances. Solutions of pure casein are not coagulated by boiling, but if heated to 130—150° in sealed tubes a coagulation is obtained.

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[1] Our knowledge of the chemistry and properties of casein are based chiefly upon the researches of Hammarsten. His papers were mostly published originally in Swedish or Latin, but are fully abstracted by himself in Maly's *Jahresbericht d. Thierchem.*, to which reference will in each case be made.

[2] For methods of conducting a complete analysis of milk see Pfeiffer, *Die Analyse der Milch*, Wiesbaden, 1887.

[3] Hammarsten, Maly's *Bericht.* Bd. VII. (1877), S. 159.

[4] Hammarsten, Maly's *Ber.* Bd. IV. (1874), S. 135.

[5] Hoppe-Seyler, *Hdbch. d. phys.-path. chem. Anal.* Aufl. IV. (1875), S. 241.

When acids are added to diluted milk to effect the precipitation of casein no precipitate is obtained until the solution has a distinctly acid reaction; this has usually been attributed to the presence in milk of potassium phosphate.[1] Hammarsten has however shewn[2] that the same holds good for solutions of casein free from this salt.

When prepared from milk by magnesium sulphate (see below), freed by ether from fats, and dissolved in water, casein possesses a specific rotatory power $(a)_D = -80°$; in dilute alkaline solutions, of $-76°$; in strong alkaline solutions, of $-91°$; in very dilute solutions, of $-87°$.[3]

Although purified casein leaves no ash-residue on ignition, Hammarsten found that it contained a *constant* and fairly large amount of phosphorus, as a mean ·847 p.c. From this fact and its behaviour towards sodium chloride in dilute solutions, he regards casein as being a nucleo-albumin[4] (see below). This view corresponds with the results previously obtained by Lubavin,[5] who found that a phosphorised (nuclein) constituent of casein is separated out as an insoluble residue during the digestion of casein with gastric juice.

According to the views of many authors[6] milk contains not one casein only, but at least two forms of proteid which pass under the one name. Hammarsten[7] has criticised these views and concludes that casein is a unitary substance, and not a mixture or compound.

Action of rennin on casein. This has been fully studied by Hammarsten, whose results may be summarised as follows: Contrary to the older views that the formation of the clot is rather of the nature of a precipitation than a true ferment action, we now know that by the action of rennin the clotting of casein is due to a specific action of the enzyme which results in the formation of a substance (tyrein) differing essentially from casein. It had been considered that the separation of the clot was due to the formation of lactic acid from milk-sugar,[1] but this is not so;[2] pure casein free from every trace of lactic acid clots with rennin. The specific action of the enzyme is further shewn by the fact that simultaneously with the formation of the clot, a by-product is formed having the properties of a soluble albumin.[3] Further, the clot is entirely different from casein: it is much less soluble in acids and alkalis than the latter,[4] always leaves as ordinarily prepared a large and constant residue of ash (calcium phosphate) on ignition, and even if it be freed from the calcium salt by special methods[5] and dissolved in dilute alkalis, is not capable of being made to yield a clot by the renewed action of rennin.

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[1] Kühne, *Lehrb. d. physiol. Chem.* 1868, S. 565.

[2] Maly's *Ber.* VII. S. 162.

[3] Hoppe-Seyler, *Hdbch.* (Ed. V.) p. 286.

[4] Maly's *Ber.* IV. (1874), S. 153.

[5] Hoppe-Seyler's *Med.-chem. Untersuch.* Hf. IV. (1871), S. 463.

[6] Millon u. Commaille, *Zt. f. Chem.* 1865, S. 641. *Compt. Rend.* T I. (1865), pp. 118, 859, T. II. p. 221. Selmi, *Ber. d. d. chem. Gesell.* Bd. VII. (1874), S 1463. Danilewsky u. Radenhausen. See Maly's *Ber.* Bd. X. (1880), S. 186. *Zt. f. physiol. Chem.* Bd. VII. (1883), S. 427. Struve, *Jn. f. prakt. Chem.* (2) Bd. XXIX. S. 71.

[7] Maly's *Ber.* Bd. V. (1875), S. 119, Bd. VI. (1876), S. 13 *Zt. f. physiol. Chem.* Bd. VII. (1883), S. 227.

It may be remarked here that no efforts to obtain a 'curd' from milk by purely chemical means, such as the addition of acids or neutral salts, have resulted in the production of a substance which by further treatment can be made to yield a typical ripening 'cheese.' The latter can only be made by the use of rennin.

The calcium salt plays an all-important part in the clotting of casein. Casein freed from this salt and dissolved in dilute alkali will not yield a clot; dialysed milk similarly yields no clot, but if the dialysate be concentrated and added to the milk it now clots on the addition of rennin. When *pure* casein is dissolved in lime-water and neutralised with phosphoric acid it now clots with rennin. The action of the salt in the whole process appears to be that it determines not so much the action of the ferment on the casein, but rather the subsequent separation from solution of the altered product.[6] Neither is the calcium salt alone essential, for it may be replaced, but with less efficient results, by the similar salts of magnesium, barium, and strontium.[7]

The question as to the identity or the reverse of casein and alkali-albumin as obtained by the action of alkalis on other proteids has given rise to much controversy. Some authors have considered them to be identical,[1] but that they are not so is sufficiently shewn by the following facts. Solutions of alkali-albumin cannot be made to clot by the action of pure rennin. If milk sugar be added to the solution and *impure* rennet, i e. extract of the mucous membrane containing rennin, be allowed to act upon it, in some cases a separation of the alkali-albumin may take place, owing to the formation of lactic acid which then precipitates the albumin. In the absence of the milk sugar no change is produced which can in any way be regarded as analogous to the clotting of casein. When milk is clotted the separation of the casein is so complete that none is found in the 'whey,' and Hammarsten has shewn that if alkali-albumin be added to milk and the mixture be then clotted, alkali-albumin may be obtained from the whey on breaking up the curd. It has further been shewn[2] that although casein is very resistant to the action of acids, it may by treatment with them be converted into acid-albumin with complete loss of all clotting powers, and still more readily into alkali-albumin by the action of alkalis.

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[1] Soxhlet, *Jn. f. pr. Chem.* Bd. VI. (1872), S. 1.

[2] Hammarsten, Maly's *Ber.* II. (1872), S. 118, IV. (1874), S. 135. Heintz, *Jn. f prakt. Chem.* N. F. Bd. VI. (1872), S. 374.

[3] Hammarsten. See also Köster (Swedish) in Maly's *Ber.* Bd. XI. (1881), S. 14.

[4] Al. Schmidt, *Beitr. z. Kennt. d. Milch*, Dorpat, 1874.

[5] Köster, *loc. cit.* S. 14.

[6] For further observations on the influence of salts on the clotting of milk and casein see Ringer, *Jl. of Physiol.* Vol. XI. (1891), p. 464, XII. (1891), p. 164.

[7] Lundberg (Swedish). See Maly's *Ber.* Bd. VI. (1876), S. 11

A further difference of the two substances was urged by Zahn on the basis of his experiments on the filtration of milk through porous earthenware (battery-cells).[3] He found that solutions of alkali-albumin pass through the walls of the cells as rapidly as do solutions of serum-albumin; when milk however is filtered by this method, casein does not pass, and the filtrate consists of water, salts, and the coagulable proteid of the milk. Whether this indicates any difference between the two substances is however doubtful, for it is still an open question whether casein is truly in solution in milk. Further it is stated that the casein also passes into the filtrate if the filtration is prolonged,[4] and Soxhlet states that if finely divided (emulsified) fat be suspended in a solution of alkali-albumin the filtration of this substance is rendered as impossible as that of casein in milk.

The crucial distinction between the two substances is the fact that casein can be clotted by rennin with simultaneous formation of a soluble proteid by-product, whereas no true clot can ever be obtained from ordinary alkali-albumin.

After the removal of casein from milk by precipitation, the filtrate contains a small amount of coagulable proteid, sometimes spoken of as 'lactalbumin,' closely resembling serum-albumin in its general properties, but differing slightly as to its specific rotatory power and the temperature at which it coagulates when heated.[5]

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[1] Soxhlet, *loc. cit.*

[2] Lundberg, *loc. cit.*

[3] Zahn, Pflüger's *Arch.* Bd. II. (1869), S. 598.

[4] Schwalbe, *Centralb. f. d. med. Wiss.* 1872, S. 66.

[5] Sebelien, *Zt. f. physiol. Chem.* Bd. IX. (1885), S. 445, XIII. (1889), S. 135. Eugling, see Maly's *Bericht.* Bd. XV. (1885), S. 183. Halliburton, *Jl. of Physiol.* Vol. XI. (1890), p. 451.

In addition to these, according to the older views, milk, even when quite fresh, frequently contained traces of a proteid which, since it yielded the biuret reaction, was usually spoken of as a peptone, and was by some observers called 'lactoprotein.[1] It was stated to increase in amount in the milk on standing for some time, and more especially if warmed to 40°, and to be considerably increased during the clotting induced by rennin.[2] Recent researches have however shewn that perfectly fresh milk contains no substance which yields a biuret reaction, its presence being due to its formation during the processes employed in its separation.[3] If the milk undergoes an acid (lactic) fermentation a substance may now be obtained from it which yields a biuret reaction, but is not a true peptone, but a primary albumose.

When milk is kept for some time at a temperature above 50° and below its boiling point, a firm skin is formed over its surface composed largely of casein.[4] Its formation is not to be regarded as being specially characteristic of milk, for pure casein dissolved in dilute alkalis exhibits the same phenomenon, as also do alkali-albumin, chondrin, gelatin, and the filtrate from 1 p.c. starch when it is concentrated on a water-bath. Its formation is probably due to the rate of evaporation from the surface of the milk being more rapid than the fluid diffusion into the upper layer;[5] and in accordance with this it is found that its appearance is considerably facilitated by blowing a rapid stream of air or any indifferent gas, such as carbonic oxide, over the surface of the warmed milk.

Our knowledge of the chemical properties of casein as already described is based entirely upon researches carried out upon the milk of cows. There is no reason to suppose that all that has been said does not apply equally well to the milk of other animals. Nevertheless human milk shews, apart from the difference of composition (see § 513), certain differences from cow's milk, which are due to a distinct but characteristic difference in the reactions of the casein contained in each.[6] This is shewn by the following facts. (1) Human milk clots less firmly than cow's milk, and sometimes not at all with rennin. (2) The casein in human milk, on the addition of acetic acid, yields a very imperfect precipitate which is finely flocculent, almost granular as compared with the compact and coarsely flocculent precipitate yielded by cow's milk. (3) The casein in human milk is, as already stated, very incompletely precipitated by the addition of acids, and can only be completely precipitated by saturation with magnesium sulphate.[1] (4) Casein from human milk is less soluble in water than is that of the cow.

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[1] Hammarsten, Maly's *Bericht.* Bd. VI. (1876). S. 13. Palm (Russian), *Ibid.* Bd. XVI. (1886), S. 143. For other references see Halliburton, *loc. cit.* p. 459.

[2] Hoppe-Seyler, *Handbuch d. phys.-path. chem. Anal.* 1883, S. 480.

[3] Neumister, *Zt. f. Biol.* Bd. XXIV. (1888), S. 280.

[4] Sembritzky, Pflüger's *Arch.* Bd. XXXVII. (1885), S. 460. See also Maly's *Ber.* Bd. XVII. (1887), S. 157.

[5] Hoppe-Seyler, Virchow's *Arch.* Bd. XVII. (1859), S. 420.

[6] Simon, *Animal Chemistry* (Sydenham Soc.), Vol. II. 1846, p. 53. Also in "Die Frauenmilch u. s. w." Berlin, 1838. Biedert, Virchow's *Arch.* Bd. LX. (1874), S. 352. Biel, see Abst. in Maly's *Ber.* Bd. IV. (1874), S. 166. Langgaard, Virchow's *Arch.* Bd. LXV. (1875), S. 352.

The primary digestive products 'caseoses' obtained by the action of pepsin on casein have been described and studied by Chittenden and Painter.[2]

Class III. *Globulins.*

Besides the derived albumins there are a number of native proteids which differ from the albumins in not being soluble in distilled water; they need for their solution the presence of an appreciable, though it may be a small, quantity of a neutral saline substance such as sodium chloride. Thus they resemble the albuminates in not being soluble in distilled water, but differ from them in being soluble in dilute sodium chloride or other neutral saline solutions.[3] Their general characters may be stated as follows.

They are insoluble in water, soluble in dilute (1 p.c.) solutions of sodium chloride; they are also soluble in dilute acids and alkalis, being changed on solution into acid- and alkali-albumin respectively unless the acids and alkalis are exceedingly dilute and their action is not prolonged. The saturation with solid sodium chloride or other neutral salts of their saline solutions precipitates most members of this class.

1. **Crystallin.** (*Globulin of the crystalline lens.*)

This form of globulin is usually regarded as identical with vitellin. It is however convenient to treat it separately, inasmuch as it can be prepared in a pure form, whereas vitellin has not as yet been obtained free from lecithin (see below).

Preparation.[4] Crystalline lenses, in which it occurs to the extent of 24·62 p.c., are rubbed up in a mortar with a little fine sand and a few crystals of rock salt; the mass is then extracted with water and filtered. The filtrate contains the crystallin and some serum-albumin. The former is separated from the latter by copious dilution with distilled water and passing a current of carbonic anhydride through the diluted mixture, whereupon the crystallin is precipitated.

A dilute saline solution of this proteid coagulates at 75°.

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[1] Makris, *Inaug.-Diss.*, Strassburg, 1876. See Maly's *Ber.* Bd. VI. (1876), S. 113

[2] *Stud. Lab. Physiol. Ch. Yale Univ.* Vol. II. (1887), p. 156.

[3] But see Nikoljukin (Russian), Abst. in Maly's *Ber.* Bd. XVIII. (1888), S. 5.

[4] Laptschinsky, Pflüger's *Arch.* Bd. XIII. (1876), S. 631.

Béchamp has recorded[1] some determinations of its specific rotatory power which must however be accepted with caution.

2. **Vitellin.**[2]

This constitutes the characteristic proteid constituent of egg-yolk and is also largely present in caviar. Some at least of the globulins present in vegetable protoplasm, and more particularly in the crystals of the aleurone grains, appear to be identical in their general properties and reactions with vitellin. As obtained in conjunction with some lecithin by exhaustion of egg-yolk with ether, it consists of a white, pasty, granular mass, insoluble in water, readily soluble in solutions of sodium chloride which may be easily filtered. Unlike other true globulins it cannot be precipitated from this solution by saturation with sodium chloride. Its saline solutions (10 p.c. NaCl) are coagulated by heating to 75°. It is readily soluble in 1 p.c. sodium carbonate, is incompletely precipitated from this solution by dilution with water, but fairly completely by the additional passing of a stream of carbonic acid gas through the diluted solution.

As has been already stated, vitellin is associated in egg-yolk with lecithin and (?) nuclein. It has not as yet been obtained free from admixture with the former, and a theory has been advanced that it is really a complex substance resembling in this respect haemoglobin, which on treatment with alcohol splits up into coagulated proteid and lecithin. It is possible that pure vitellin free from lecithin might be obtained by prolonged extraction with ether in a Soxhlet or other form of apparatus.

Frémy and Valenciennes have described[3] a series of proteids, viz. ichthin, ichthidin &c. derived from the eggs of fishes and amphibia. They appear to be closely related to vitellin but have not been sufficiently investigated.

The primary products obtained from vitellin by the digestive action of pepsin have been examined and described by Neumeister.[4]

Preparation. Egg-yolk is extracted with successive portions of ether as long as the residue yields any colour to the solvent. The pasty residue thus obtained is dissolved in a minimal amount of 8—10 p.c. sodium chloride solution, precipitated from this by the addition of an excess of water, and purified by resolution in the salt and reprecipitation by the addition of water. The operations must be conducted as rapidly as possible since the prolonged action of water renders the vitellin insoluble in saline solutions. If any attempt is made to separate the vitellin from lecithin residues by means of alcohol it is at once converted into ordinary coagulated proteid.

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[1] *Compt. Rend.* T. xc. (1880), p. 1255.

[2] Dumas et Cahours, *Ann. Chem. et Phys.* (3) T. vi. p. 422. Hoppe-Seyler, *Med.-chem. Unters.* (Tübingen), Hft. 2 (1867), S. 215. Weyl, *Arch. f. Physiol.* Jahrg. 1876, S. 546. Pflüger's *Arch.* Bd. xii. (1876), S. 635. *Zt. f. physiol. Chem.* Bd. i. (1877), S. 72.

[3] *Compt. Rend.* T. xxxviii. pp. 469, 525, 570.

[4] *Zt. f. Biol.* Bd. xxiii. (1887), S. 402. Cf. Chittenden and Hartwell, *Jl. of Physiol.* Vol. xi. (1890), p. 441.

3. **Paraglobulin.** (*Serum-globulin.*)[1]

This proteid occurs most characteristically in blood-serum (also in lymph), in amounts now known to be much larger than was at one time supposed, and thus constituting about one-half of the total proteids of the serum.[2]

Preparation.[3] The older methods consisted in (1) diluting serum ten-fold with water and passing a prolonged current of carbonic acid gas; (2) saturating serum with sodium chloride. The amount of precipitate thus obtained represents only a small part of the total paraglobulin present in the serum,[4] and the only satisfactory method of preparing it pure and in considerable quantity is as follows: (3) serum is saturated at 30° with magnesium sulphate, by means of which paraglobulin is quantitatively precipitated. The precipitate collected by filtration is distributed through a small volume of a saturated solution of the magnesium salt, collected on a filter and washed with saturated solution of $MgSO_4$. By this means it is separated from the larger part of the serum-albumin.

To effect its final and complete separation from this latter proteid, two methods may be adopted. (*a*) The precipitate is dissolved in water, then largely diluted and the paraglobulin further separated out by passing a stream of CO_2. (β) The precipitate is dissolved as before in water, the paraglobulin again salted out by $MgSO_4$, this process repeated several times, and the final product separated from the magnesium salt by dialysis.[5]

[1] This is the substance to which Al. Schmidt gave the name of fibrino-plastin. (*Arch. f. Anat. u. Physiol.* Jahrg. 1861, Sn. 545, 675. *Ibid.* 1862, Sn. 428, 533. Pflüger's *Arch.* Bd. VI. (1872), S. 413. *Ibid.* XI. (1875), Sn. 291, 526.) It had previously been described under the name 'serum-casein' by Panum. (Virchow's *Arch.* Bd. IV. (1852), S. 17.) The name paraglobulin is due to Kühne (*Lehrbuch* 1868, Sn. 168, 175). It is now generally and most appropriately known by the latter name, or that of serum-globulin, as suggested by Hoppe-Seyler.

[2] Hammarsten, Pflüger's *Arch.* Bd. XVII. (1878), S. 413. Salvioli, *Arch. f. Physiol.* 1881, S. 269.

[3] Gamgee, *Physiol. Chem.* Vol. I. p. 37.

[4] Hammarsten, *loc. cit.* Heynsius, Pflüger's *Arch.* Bd. XII. (1876), S. 549.

[5] Hammersten, *loc. cit.* Also Pflüger's *Arch.* Bd. XVIII. (1878), S. 38. *Zt. f. physiol. Chem.* Bd. VIII. (1883), S. 467. Denis had previously used magnesium sulphate for the quantitative separation of serum-globulins ("Mémoire sur le Sang, 1859"), but Hammarsten rediscovered the general method independently, and ap-

Pure paraglobulin is insoluble in water. If dissolved in a *minimal amount* of alkali it is precipitated by ·03 to ·5 p.c. of NaCl. On the addition of more than ·5 p.c. of the salt it goes again into solution and does not begin to be reprecipitated on the addition of more salt until at least 20 p.c. NaCl has been added. It is not completely precipitated by saturation of its solutions with NaCl (Hammarsten). Its dilute saline solutions coagulate on heating to 75°.[1] Dissolved in dilute solutions of NaCl or $MgSO_4$ its specific rotatory power is stated to be $(a)_D = -47{\cdot}8°$.[2]

Paraglobulin occurs in smaller amounts ($\frac{1}{3}$—$\frac{1}{2}$) in chyle, lymph, and serous fluids. Hammarsten by means of saturation with $MgSO_4$ was the first to shew that hydrocele fluids frequently contain paraglobulin, thus largely shaking the importance of Al. Schmidt's views as to the part it plays in the process of blood-clotting.

Globulins which are not regarded as differing essentially from paraglobulin are also stated to occur in urine.[3]

Cell-globulins. Halliburton has described under this name[4] some forms of globulin which occur in lymph-corpuscles and may be extracted from them by solutions of sodium-chloride. Of these one, cell-globulin-*α*, occurs in minute quantities only and is characterised by coagulating at 48—50°. The other, cell-globulin-*β*, is more copiously present in the corpuscles and coagulates in dilute saline solutions at 75°. The latter resembles paraglobulin very closely in properties other than the identity of their temperatures of heat coagulation in dilute saline solution, e. g. precipitability, &c. He considers that cell-globulin-*β* differs from true paraglobulin, or plasma-globulin as he terms it, by possessing the power of hastening the clotting of diluted salt-plasma, and he regards the so-called 'fibrin-ferment' as identical with cell-globulin-*β* and arising from the disintegration of leucocytes.

The proteid constituent of the stroma of red blood-corpuscles consists chiefly of a globulin usually regarded as identical with paraglobulin, since its saline solutions coagulate at 75° and it is precipitated from the same by saturation with sodium chloride and a current of carbonic anhydride.[5] Halliburton considers it to be identical with

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plied it somewhat differently to Denis. On the use of ammonium sulphate for separating globulins and serum-albumin see Michailow (Russian), Abst. in Maly's *Bericht.* Bd. XIV., XV. (1884–5), Sn. 7, 157. Pohl, *Arch. f. exp. Path. u. Pharm.* Bd. XX. (1886), S. 426.

[1] Halliburton, *Jl. of Physiol.* Vol. V. (1883), p. 157.

[2] Frédéricq, *Arch. de Biol.* T. I. (1880), S. 17. *Bull. Acad. roy. de Belgique* (2), T. IV. (1880), No. 7. (See Maly's *Bericht.* 1880, S. 171.)

[3] Lehmann, Virchow's *Arch.* Bd. XXXVI. (1866), S. 125. Edlefsen, *Arch. f. klin. Med.* Bd. VII. (1870), S. 67. Also *Centralb. f. med. Wiss.* 1870, S. 367. Senator, Virchow's *Arch.* Bd. LX. (1874), S. 476. Heynsius, Pflüger's *Arch.* Bd. IX. (1874), S. 526 (foot-note). Führy-Snethlage, *Arch. klin. Med.* Bd. XVII. (1876), S. 418.

[4] *Proc. Roy. Soc.* Vol. XLIV. (1888), p. 255. *Jl. of Physiol.* Vol. IX. (1888), p. 235.

[5] Hoppe-Seyler, *Physiol. Chem.* S. 391. Kühne, *Lehrbuch,* S. 193. Wooldridge, *Arch. f. Physiol.* Jahrg. 1881, S. 387. Hoppe-Seyler, *Zt. f. physiol. Chem.* Bd. XIII. (1889), S. 477.

cell-globulin-β, and accounts thus for the earlier statements as to the fibrinoplastic properties of the stroma-globulins.[1]

4. Fibrinogen.[2]

This globulin occurs in blood-plasma together with paraglobulin and serum-albumin. During blood-clotting it is converted largely, if not entirely, into fibrin (but see below). It is also found in chyle, serous fluids and transudations, more particularly in hydrocele fluids.[3]

In its *general* reactions it resembles paraglobulin but is markedly distinguished from the latter by the following characteristics. (1) As it occurs in plasma[4] or in dilute solutions of sodium chloride (1—5 p.c.), it coagulates at 55—56°. (2) It is very readily precipitated by the addition of sodium chloride to its saline solutions until the whole contains 16 p.c. NaCl, whereas paraglobulin is not appreciably precipitated until at least 20 p.c. of the sodium salt has been added.

Preparation.[5] Salted plasma, obtained by centrifugalising blood whose coagulation is prevented by the addition of a certain proportion of magnesium sulphate, is mixed with an equal volume of a saturated (35·87 p.c. at 14° C.)[6] solution of sodium chloride; the fibrinogen is thus precipitated while the paraglobulin remains in solution. The adhering plasma may be removed by washing with a solution of sodium chloride, and the fibrinogen finally purified by being several times dissolved in and reprecipitated by sodium chloride.

Hammarsten's statements as to the nature and properties of fibrinogen have been the subject of much controversy between himself, Al. Schmidt, and Wooldridge.

When a fluid containing purified fibrinogen is made to yield fibrin by the action of fibrin-ferment, the amount of fibrin formed

[1] *Jl. of Physiol.* Vol. x. (1889), p. 532.

[2] Hammarsten, *Nov. Act. Reg. Soc. Sci.*, Upsala, Vol. x. 1, 1875. Maly's *Bericht.* vi. (1876), S. 15. Pflüger's *Arch.* Bd. xiv. (1877), S. 211; xix. (1879), S. 563; xxii. (1880), S. 431; xxx. (1883), S. 437. Maly's *Bericht.* xii. (1882), S. 11. Al. Schmidt, Pflüger's *Arch.* Bd. vi. (1872), S. 413; xi. (1875), S. 291; xiii. (1876), S. 146. "Lehre von den ferment. Gerinnungserscheinungen u. s. w.," Dorpat, 1877. Wooldridge, *Jl. of Physiol.* Vol. iv. (1883), pp. 226, 367. *Arch. f. Physiol.* 1883, S. 389; 1884, S. 313; 1886, S. 397. *Proc. Roy. Soc.* Vol. lxii. (1887), p. 230. Ludwig's *Festschrift*, 1887, S. 221. *Zt. f. Biol.* Bd. xxiv. 1888, S. 562. *Arch. f. Physiol.* 1888, S. 174. *Jl. Physiol.* Vol. x. (1889), p. 329.

[3] Hammarsten, Maly viii. (1878), S. 347.

[4] Frédéricq, *Ann. Soc. de Méd. Gand*, 1877. *Arch. d. Zool. Exp.*, 1877, No. 1. *Bull. de l'Acad. roy. de Belgique*, T. lxiv. (1877), No. 7. "Recherches sur la constitution du plasma sanguin." Paris, 1878.

[5] Hammarsten, *loc. cit.* passim. Gamgee, *Physiol. Chem.* Vol. i. p. 41.

[6] Poggiale, *Ann. Chim. Phys.* (3), Vol. viii. p. 469.

is always less than that of the fibrinogen which disappears at the same time.[1] The deficit thus observed is at least partly accounted for by the simultaneous appearance of a globulin which coagulates, when heated in saline solution, at 64°. Although at first sight it seems very tempting to regard the process of fibrin-formation from fibrinogen as partaking of the nature of a hydrolytic (?) cleavage of which this globulin is one product, this view is not as yet established. Hammarsten considers it is more probable that the globulin really represents a portion of the fibrin which has gone into solution during its formation, basing his views on the earlier work of Denis,[2] who showed that under special circumstances a form of fibrin may be obtained which is soluble in solutions of sodium chloride, the solution coagulating at 60—65° (see below, p. 33). Al. Schmidt holds that Hammarsten's fibrinogen as coagulating at 55° is in reality a sort of modified or "nascent" fibrin and not truly a globulin.

The viscid secretion of the vesicula seminalis of the guinea-pig is very rich in proteids and possesses the power of clotting. The proteid which it contains is not in all respects a typical globulin, but in many ways it resembles fibrinogen. When dissolved in a little lime-water it coagulates when heated to 55°. The secretion itself clots readily and firmly on the addition of a small quantity of the aqueous extract of a blood clot.[3]

The fibrinogen of invertebrate blood yields fibrin by the action of fibrin ferment, but differs from vertebrate fibrinogen by coagulating at 65° when heated.[4]

5. **Myosin.**

When an irritable contractile muscle passes into rigor, the substance of which the muscle-fibres are chiefly composed undergoes a change, analogous to the clotting of blood-plasma, which results in the formation of a clot of myosin.[5] By appropriate methods (see § 59) the muscle-fibres may be broken up and their contents obtained as a viscid, slightly opalescent fluid (muscle-plasma), which filters with difficulty and clots at temperatures above 0°. This muscle-plasma may be diluted with solutions of varying strengths of several neutral salts, whereby its clotting may be delayed, and the nature and phenomena of the processes involved in the clotting investigated along the lines previously employed in the elucidation of the phenomena of the clotting of blood-plasma.[1] The more important facts which have thus been made out may be briefly summarised as follows. Muscle-plasma contains a globulin-forerunner of myosin ('myosinogen') which resembles fibrinogen in coagulating at 56°. This proteid is converted into myosin on the occurrence of clotting by the action of a specific ferment, which is regarded as being closely related to, if not identical with, an albumose (see below). The serum, which is left in small quantities only after the formation of the clot, contains proteids which coagulate at 47°[2] (paramysinogen) 63°, (myoglobulin) 73°, an albumin closely resembling serum-albumin.

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[1] Hammarsten, Pflüger's *Arch.* Bd. xxx. (1883), Sn. 459, 465, 475.

[2] "Nouvelles études chimiques, etc." Paris, 1856, p. 106. "Mémoire sur le sang," 1859.

[3] Landwehr, Pflüger's *Arch.* Bd. xxiii. (1880), S. 538.

[4] Halliburton, *Jl. of Physiol.* Vol. vi. (1884), p. 321.

[5] Kühne, "Das Protoplasma," 1864. *Lehrbuch,* S. 272.

Preparation.[3] (1) Finely chopped muscle-substance is washed rapidly with cold water, to remove serum-albumin and colouring matters (haemoglobin), the residue is squeezed out in linen, and extracted for at least 24 hours with 10 p.c. solution of NH_4Cl in which myosin is readily soluble. The extract is now filtered first through muslin and then through paper; the filtrate is a more or less viscid and opalescent solution of myosin. From this the myosin may be prepared in a pure condition by allowing its solution in the ammonium salt to drop into a large excess of distilled water. The myosin gradually settles out in a flocculent mass, which may be further purified by resolution in a minimal amount of neutral salt and reprecipitation by pouring into an excess of distilled water. This purification must be conducted rapidly and at low temperatures, for myosin is somewhat readily altered by the prolonged action of water and becomes insoluble in saline solutions.[4] (2) The finely chopped and washed muscle is divided into two equal portions: to one of these *very dilute* (deci-normal) hydrochloric acid is *carefully* added until a distinct acid reaction is obtained as shewn by tropaeolin OO (see above, p. 18). The two portions are then intimately mixed together, allowed to stand some time, strained through muslin, filtered and the myosin precipitated from the filtrate by careful neutralisation with very dilute alkali or lime-water.

Apart from the general reactions which characterise myosin as a globulin, it is distinguished by the low temperature (55—56°) at which its saline solutions constantly coagulate. It leaves a large ash residue on incineration, consisting chiefly of salts of lime. As already stated, it is converted into an insoluble proteid by the prolonged action of water, and into syntonin by the action of acids. These substances are stated to be capable of reconversion into myosin (see above, p. 17). It is also stated[5] that if myosin is dissolved in NaCl or $MgSO_4$ (10 and 5 p.c. respectively) it yields a renewed clot on mere dilution with water.

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[1] Halliburton, *Jl. of Physiol.* Vol. VIII. (1887), p. 133.
[2] Cf. Demant, *Zt. f. physiol. Chem.* Bd. III. (1879), S. 241; IV. (1880), S. 384.
[3] Danilewsky, *Zt. f. physiol. Chem.* Bd. V. (1881), 158.
[4] Weyl, *Zt. f. physiol. Chem.* Bd. I. (1877), S. 77.
[5] Halliburton, *loc. cit.* p. 148.

According to Nasse[1] myosin constitutes the anisotropous substance (see above § 56) of the unaltered muscle-fibre, and the activity of contraction is inversely proportional to the amount of this substance which is present in the fibres of different animals.

Globulins to which the name of myosin is applied are described as occurring in vegetable protoplasm[2] and in the cells of the liver.[3]

Myosin is readily digested by pepsin, more slowly by trypsin. The primary products arising from the digestive action of the former enzyme have been studied by Kühne and Chittenden.[4]

6. **Globin.**

When haemoglobin is allowed to undergo decomposition spontaneously by exposure to the air an insoluble proteid is obtained of which very little is known, but to which the name of globin was given by Preyer.[5] It appears to be perhaps an outlying member of the globulin class of proteids, but unlike a true globulin is scarcely soluble in dilute acids and imperfectly soluble in alkalis and solutions of sodium chloride. It is converted into acid and alkali-albumin by the action of strong acids and alkalis respectively, and is stated to yield no trace of ash on incineration.

Class IV. *Fibrin.*

This proteid is ordinarily obtained by 'whipping' blood with a bundle of twigs until clotting is complete; the fibrin which adheres to the twigs is then washed in a current of water until all the haemoglobin of the entangled corpuscles is removed and it is now quite white. The washing is greatly facilitated if the fibrin is very finely chopped before it is washed, and if it is frequently kneaded and squeezed with the hand during the washing. In this way it may be obtained quite white in a few hours. The washing is also much facilitated if the blood is mixed with an equal bulk of water before it is whipped. It is obvious that fibrin prepared by the above method must be in an extremely impure condition, for it contains a not inconsiderable admixture of the remains of the white corpuscles and the stromata of the red.[1] It can only be prepared pure during the clotting of either filtered or centrifugalised iced-plasma or salt-plasma, or by the action of purified fibrin-ferment on pure fibrinogen. In accordance with this, fibrin as ordinarily obtained leaves a variable amount of granular residue which contains phosphorus during its digestion by pepsin. No such residue is observed when fibrin from filtered plasma is digested with pepsin (see below, p. 42), but in no other essential respect does the one fibrin differ from the other.

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[1] "Anat. u. Physiol. d. Muskelsubst." Leipzig, 1882. *Biol. Centralb.* Bd. II. (1882–3), S. 313. *Zt. f. physiol. Chem.* Bd. VII. (1882), S. 124.

[2] Weyl, *Zt. physiol. Chem.* Bd. I. (1877), S. 96.

[3] Plósz, Pflüger's *Arch.* Bd. VII. (1873), S. 377.

[4] *Zt. f. Biol.* Bd. XXV. (1889), S. 358. See also Chittenden and Goodwin, *Jl. of Physiol.* Vol. XII. (1891), p. 34.

[5] "Die Blutkrystalle," Jena, 1871, S. 166.

Fibrin, as ordinarily obtained, exhibits a filamentous structure, the component threads possessing an elasticity much greater than that of any other known solid proteid.

If allowed to form gradually in large masses, the filamentous structure is not so noticeable, and it resembles in this form pure india-rubber. Such lumps of fibrin are capable of being split in any direction, and no definite arrangement of parallel bundles of fibres can be made out.

Fibrin is insoluble in water and *dilute* saline solutions. It is also ordinarily insoluble in dilute acids (HCl) if their action takes place at ordinary temperatures and is not prolonged, merely becoming swollen and transparent in the acid and returning to its original state if the acid is removed by an excess of water or careful addition of an alkali. By prolonged action at ordinary temperatures, or a shorter action at 40°, the fibrin is profoundly changed and certain forerunners of the peptones which may be finally formed (at 40°) are produced. It is similarly insoluble in dilute alkalis and ammonia, but passes more readily into solution in these reagents, if their action is prolonged or the temperature is raised, than is the case with dilute acids. The behaviour .of fibrin towards solutions of neutral salts is peculiar and important. As already stated, fibrin prepared by simply whipping blood is insoluble in dilute saline solutions. But its solubility is dependent upon the conditions under which it is separated out from the blood. In accordance with this, Denis[2] described three forms of fibrin to which he gave the names of 1. Fibrine concrète modifiée. 2. Fibrine globuline. 3. Fibrine concrète pure. The first is what we now know as ordinary fibrin obtained by whipping arterial blood (human in Denis' work). The second he obtained by the spontaneous clotting of human venous blood, and this readily swells up to a slimy mass in 10 p.c. NaCl. The third he prepared by 'whipping' human venous blood under certain precautions, and found it to be soluble in dilute salt solution by one or two hours' treatment with the same at 40°. Quite apart from Hammarsten's partial confirmation of Denis' statements there is but little reason for doubting the accuracy of so careful a worker.

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[1] Hammarsten, Pflüger's *Arch.* Bd. xxii. (1880), S. 481; xxx. (1883), S. 440.

[2] For reference see p. 30.

The possible solubility of fibrin under certain conditions in saline solutions of moderate strength obtained considerable importance in the controversy between Schmidt and Hammarsten as to the nature of the processes involved in the clotting of blood. When on the other hand fibrin is subjected to the prolonged action of more concentrated (10 p.c.) solutions of neutral salts, and the salt solution is frequently renewed, the fibrin may be finally completely dissolved, being converted into members of the globulin class.[1] Most observers agree that the globulin thus chiefly formed coagulates at 55—56°. Green obtained in addition one coagulating at 59—60°, the two differing further in their solubilities in 1 and 10 p.c. solutions of NaCl. These changes are brought about by the salts in the entire absence of any putrefactive phenomena, and the resulting globulins cannot be made to yield fibrin again by any treatment with fibrin-ferment.

When fresh unboiled fibrin is simply washed till it is white and digested with pure active trypsin, it is largely converted into coagulable proteids during the initial stages of the ferment action.[2] These proteids are characteristically globulins and one is closely related to paraglobulin, as judged of by its coagulating in saline solutions at 75° and possessing a specific rotatory power (in 10 p.c. NaCl) of $(\alpha)_D = -48{\cdot}1^\circ$.[3] The second globulin product of the ferment action coagulates at 55—56°, and in this respect more closely resembles fibrinogen.[4] Whether the whole of the globulin thus obtained is a product of the conversion of the fibrin, or whether a portion of it is due to globulin existing as such in the raw fibrin, is not yet stated. Similar globulins are produced by the action of pepsin in its earlier stages on raw fibrin. If the fibrin is boiled or treated for some time with alcohol before digestion with either of the above enzymes, mere traces, if any, of these globulins are obtained.

The purest fibrin always leaves a small but fairly constant ash-residue on incineration. Of the inorganic constituents of which this residue is composed it is probable that sulphur is the only element which enters essentially into the composition of the fibrin.

When boiled in water or treated for some time with alcohol it loses its elasticity, becomes much more opaque, is much less soluble in the various reagents which dissolve the original fibrin with comparative ease, is attacked with much greater difficulty by pepsin and trypsin, and is in fact indistinguishable from all other coagulated proteids.

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[1] Green, *Jl. of Physiol.* Vol. VIII. (1887), p. 373. Limbourg, *Zt. f. physiol. Chem* Bd. XIII. (1889), S. 450. The latter contains a complete list of references to the literature of the subject excepting Plósz, Pflüger's *Arch.* Bd. VII. (1873), S. 382.

[2] Brücke, *Wien. Sitzber.* Bd. XXXVII. (1859), S. 131. Kühne, Virchow's *Arch.* Bd. XXXIX. (1867), S. 130. *Lehrbuch*, S. 118. Kistiakowsky, Pflüger's *Arch.* Bd. IX. (1874), S. 446.

[3] Otto, *Zt. f. physiol. Chem.* Bd. VIII. (1883), S. 130.

[4] Hasebroek, *Zt. f. physiol. Chem.* Bd. XI. (1887), S. 348. Herrmann, *Ibid.* S. 508. But see Neumeister, *Zt. f. Biol.* Bd. XXIII. (1887), S. 398. Salkowski, *Ibid.* Bd. XXV. (1889), S. 97.

A peculiar property of this body remains yet to be mentioned, viz. its power of decomposing hydrogen dioxide. Pieces of fibrin placed in this fluid, though themselves undergoing no change, soon become covered with bubbles of oxygen; and guaiacum is turned blue by fibrin in presence of hydrogen dioxide or ozonised turpentine.

When globulin, myosin, and fibrin are compared each with the other, it will be seen that they form a series in which myosin is intermediate between globulin and fibrin. Globulin is excessively soluble in even the most dilute acids and alkalis; fibrin is almost insoluble in these; while myosin, though more soluble than fibrin, is less soluble than globulin. Globulin again dissolves with the greatest ease in a very dilute solution of sodium chloride. Myosin, on the other hand, dissolves with difficulty; it is much more soluble in a 10 per cent. than in a one per cent. solution of sodium chloride; and even in a 10 per cent. solution the myosin can hardly be said to be dissolved, so viscid is the resulting fluid and with such difficulty does it filter. Fibrin again dissolves with great difficulty and very slowly in even a 10 per cent. solution of sodium chloride, and in a one per cent. solution it is practically insoluble. When it is remembered that fibrin and myosin are, both of them, the results of clotting, their similarity is intelligible. Myosin is in fact a somewhat more soluble form of fibrin, deposited not in threads or filaments but in clumps and masses.

CLASS V. *Coagulated Proteids.*

These are insoluble in water, dilute acids and alkalis, and neutral saline solutions of all strengths. In fact they are really soluble only in strong acids and strong alkalis, though prolonged action of even dilute acids and alkalis will effect some solution, especially at high temperatures. During solution in strong acids and alkalis a destructive decomposition takes place, but some amount of acid- or alkali-albumin is always produced, together with some peptone and allied substances.

Very little is known of the chemical characteristics of this class. They are produced by heating to 100° C. solutions of egg- or serum-albumin globulins suspended in water or dissolved in saline solutions; by boiling for a short time fibrin suspended in water, or precipitated acid- and alkali-albumin suspended in water. They are readily converted at the temperature of the body into peptones, by the action of gastric juice in an acid, or of pancreatic juice in an alkaline medium.

All proteids in solution are precipitated by an excess of strong alcohol. If the precipitant be rapidly removed they are again soluble in water, but if the precipitated proteids are subjected for some time to the action of the alcohol they are, with the exception of peptones, coagulated and lose their solubility. It appears, however, that the proteids contained in the aleurone-grains of plants are exceedingly resistant to this coagulating action of alcohol.[1]

CLASS VI. *Albumoses and Peptones.*

When any of the proteids already described are submitted to the digestive action of pepsin or trypsin, certain subtances are formed, in the earlier stages of the action, which are intermediate between the proteid undergoing digestion and the proteid product (peptone) which finally results from the action of the enzymes. When the digestive fluid employed is pepsin in presence of dilute (·2 p.c.) hydrochloric acid, a small portion of the proteid may be at first converted into a form of ordinary acid-albumin.[2] It is obtained by neutralising a peptic digestive mixture at an early stage of the digestion, and has been frequently and almost usually confounded with the 'parapeptone' of Meissner. As will be explained later on, the two substances are quite distinct forms of proteid. At a later stage of the digestion the first-formed acid-albumin disappears, a considerable amount of parapeptone is formed, and other products make their appearance, which are known collectively under the name of albumoses.[3] By a more prolonged action of the pepsin a considerable portion of these albumoses is further changed into the final product peptones;[4] beyond this stage no further change can be brought about by the action of pepsin. If trypsin be employed in an alkaline solution (·25 p.c. Na_2CO_3) the decomposition of the proteid is much more complicated and profound. Instead of acid-albumin a small amount of alkali-albumin makes its appearance, together with more or less (see above, p. 34) of the coagulable globulins in the earliest stages of the digestion. Albumoses speedily make their appearance, to be somewhat rapidly and it may be largely converted into peptones, of which some are in their turn partially, though never completely, converted into leucin, tyrosin, and other less well-defined crystalline products. Similar products of the decomposition of proteids may be obtained by the action of acids alone, in the absence of all enzyme, the preponderance of any one or more of the products being dependent upon the concentration of the acids, the temperature at which they are employed, and the duration of their action. Proteids may also be peptonised by means of water acting at high temperatures under considerable pressure. By employing the above means for effecting the decomposition of proteids, the products (proteid) which may be obtained, and which have of late years been very exhaustively dealt with and described by Kühne and his pupils, are numerous. It will hence conduce to clearness in the subsequent description of each separate product if this is preceded by a short statement of the views which have from time to time been held as to the general digestive changes which proteids may undergo.

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[1] Vines, *Jl. of Physiol.* Vol. III. (1880), p. 108.

[2] To this substance the name 'syntonin' was formerly applied; this term is however most appropriately used to denote that form of acid-albumin which results from the action of acids on myosin. (See above, p. 16.)

[3] Kühne, *Verhand. d. naturhist.-med. Ver. Heidelb.* N. F. Bd. I. (1876), S. 236. Schmidt-Mülheim (*Arch. f. Physiol.* 1880, S. 36) named these antecedents of the true peptones 'propeptone.' See also Virchow's *Arch.* Bd. I. (1880), S. 575. *Jahresber. d. Thierarzneischule*, Hannover, 1879–1880. *Biol. Centralb.* Bd. I. (1881–2), Sn. 312, 341, 558.

[4] Name due to Lehmann 1850, *Physiol. Chem.* (Ed. Cav. Soc.) Vol. II. p. 53. Peptones were first definitely described by Mialhe, *Jn. de Pharm. et de Chim.* (3 Ser.) T. x. 1846, p. 161.

The first distinct experimental demonstration of the solvent action of gastric juice was due to Réaumur (1752), which was followed at intervals by those of Stevens (1777), Spallanzani (1783), and Beaumont (1834). The chemical nature of the products arising from the solution was not, however, described until the year 1846 by Mialhe under the name of 'albuminose;' to these the name of peptone was subsequently given by Lehmann in 1850, and their most important properties fairly fully described by Mulder in 1858. In this same year Corvisart first published his views as to the specific proteolytic powers of pancreatic juice, and these were finally shewn to be correct by Kühne in 1867. During this latter period (1859—1862) Meissner and his pupils [1] had published the results of researches on the products which are formed during gastric digestion.[2]

Meissner's researches. When an alkali was added to the filtered fluid resulting from the acid peptic digestion of any proteid, to an amount just short of that required for exact neutralisation, a precipitate was obtained which he named *parapeptone.* In its general reactions it resembled acid-albumin or syntonin, but was distinctively characterised by its incapability of undergoing conversion into a peptone by the further action of pepsin. He pointed out at the same time that it might be digested by an infusion of the pancreas. After the removal of the parapeptone he occasionally obtained a further precipitate by the addition of acid, to not more than ·05 to ·1 p. c., to the filtrate; this substance he named *metapeptone.* He further described a residue insoluble in dilute acids, but soluble in dilute alkalis, which made its appearance during the digestion of casein, and to which he gave the name of *dyspeptone.* After the removal of the above products there still remained in solution three substances called respectively *a*-, *b*-, and *c*-peptone, and characterised as follows: —

a-peptone; precipitated by strong nitric acid and by potassium ferrocyanide in presence of *weak* acetic acid.

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[1] *Zt. f. rat. Med.* Bde. VII. S. 1; VIII. S. 280; X. S. 1; XII. S. 46; XIV. S. 303.
[2] See résumé by Lehmann in *Biol. Centralb.* Bd. IV. (1884), S. 407.

b-peptone; not precipitated by strong nitric acid nor by potassium ferrocyanide unless in presence of an excess of *strong* acetic acid.

c-peptone; not precipitated by nitric acid nor by the potassium salt, whatever be the amount of acetic acid simultaneously added.

These statements of Meissner led to considerable subsequent controversy, and the occurrence of the several products he described was, with the exception of parapeptone and *c*-peptone, denied by those who repeated his experiments. There is now but slight reason for doubting that the divergent views are due to the fact that Meissner's digestive extracts frequently contained only small amounts of pepsin, while those of subsequent observers were much more actively peptic, so that in their case several of the intermediate products described by Meissner were rapidly peptonised and thus missed. Further it was urged that Meissner's parapeptone was not a specific product of peptic action, for it was said to be identical in all its chemical properties with ordinary acid-albumin or syntonin Hence it was that Brucke,[1] opposing Meissner, put forward the view, which has since been most generally accepted, that the sole products of a peptic digestion are parapeptone and peptone, — the former being due to the action of the acid necessary for the activity of the pepsin, the latter making its appearance as the sole final specific product of the ferment's action on the first formed parapeptone. Schiff alone appears to have supported Meissner.[2]

The researches of Kühne. From what has been already said it is at once evident that Meissner's view implied a decomposition or splitting-up of the primary proteid molecule, inasmuch as he held that his parapeptone was incapable of conversion into peptone by the further action of pepsin. Brücke on the other hand regarded the process of peptonisation by gastric juice as not necessarily involving any decomposition of the proteid molecule. Kühne, impressed with the profound and obvious decomposition which trypsin brings about when it acts on proteids, reverted once more to the possibilities implied in Meissner's views. In so doing he found further confirmation of the idea that even in gastric peptonisation the proteid is not merely changed but split up, in the fact that only a portion of the gastric peptones can be made to yield leucin and tyrosin by the action of trypsin; from which it follows that during a complete gastric peptonisation at least two distinct peptones are formed. In accordance with this he assumed that the original proteid molecule must itself consist of two parts, of which each yielded its corresponding peptone during the hydration which leads to the formation of peptones.[1] He found also further confirmation of this probability in the work of Schützenberger.[2] This observer, decomposing proteids with acids at 100° C., came to the conclusion that half the proteid molecule is readily decomposable by the acids, while the other half is peculiarly resistent and is obtained in the final products as an extraordinarily indigestible but true proteid, to which he gave the characteristic name of 'hemiprotein.' Convinced thus of the double nature of the proteid molecule, and seeing but little hope of separating from each other in a mixture the two peptones which must presumably result from the gastric peptonisation of a proteid, Kühne endeavoured to establish their existence by trying to discover the primary products intermediate between the proteid and the peptones,—antipeptone on the one hand and hemipeptone on the other.[3] In this his endeavours were at once assisted by his being in possession of a large amount of a proteid identical with that first described and carefully examined by Bence-Jones, and hence called by his name.[4] A renewed examination of this substance revealed that it was capable of conversion by pepsin into a peptone which was readily further decomposed by trypsin.[5] It was in fact the product intermediate between the original proteid and the hemipeptone, and to it Kuhne gave the name of hemialbumose. It now was only necessary to obtain the corresponding albumose precursor of the antipeptone, to peptonise this, and shew that the peptone thus obtained would yield no leucin or tyrosin by even prolonged treatment with trypsin. This Kühne succeeded in doing by a fractionated peptic digestion[6] and thus established his own views, and in doing so shewed how accurate as a whole Meissner's statements were. This will be evident from the detailed description of the several products of the decomposition of proteids by pepsin, trypsin, and acids, which is given below. The fundamental notion, then, of Kühne's view is that an ordinary native albumin or fibrin contains within itself two residues, which he calls respectively an anti-residue and a hemi-residue The result of either peptic or tryptic digestion is to split up the albumin or fibrin, and to produce on the part of the anti-residue antipeptone, and on the part of the hemi-residue hemipeptone, the latter being distinguished from the former by its being susceptible of further change by tryptic digestion into leucin, tyrosin, &c., each peptone being preceded by a corresponding anti- or hemi-albumose. Antipeptone remains as antipeptone even when placed under the action of the most powerful trypsin, provided putrefactive changes do not intervene. Kühne's views may be conveniently exhibited in the accompanying tabular forms.

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[1] *Sitzb d. Wien. Akad.* Bd. XXXVII. (1859), S. 131; XLIII. (1861), S. 601.

[2] *Leçons sur la digestion*, 1867, T. I. p. 407; II. p. 12.

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[1] *Verhandl. d. naturhist.-med. Ver.* Heidelberg, N. F. Bd. I. (1876), S. 236.

[2] *Bull. de la Soc chim.* Paris, T. XXIII. (1875), pp. 161, 193, 216, 242, 385, 433. T. XXIV. pp. 2, 145. See abst. Maly's *Jahresb.* Bd. V. (1875), S. 299.

[3] The name 'hemipeptone' was given in order to convey the idea that it is the peptone formed from one half of the original proteid molecule 'antipeptone' on the other hand that it is that form of peptone which withstands or is opposed to (ἀντί) any further decomposing action of the agents which led to its appearance.

[4] *Phil. Trans. Roy. Soc.* Pt. I. 1848. *Ann. d. Chem. u. Pharm.* Bd. LXVII. (1884), S. 97.

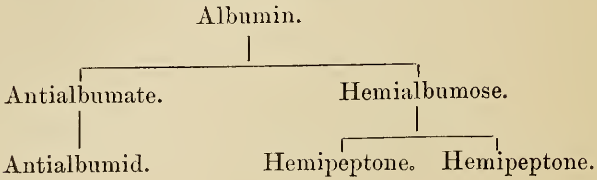
[5] Kühne, *Zt. f. Biol.* Bd. XIX. (1883), S. 209.

[6] Kühne u. Chittenden, *Ibid.* S. 171.

Decomposition of Proteids by Acids.

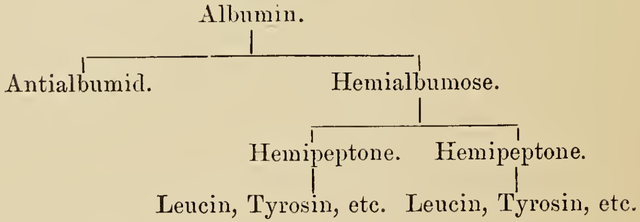
1.

By ·25 p. c. HCl at 40° C.

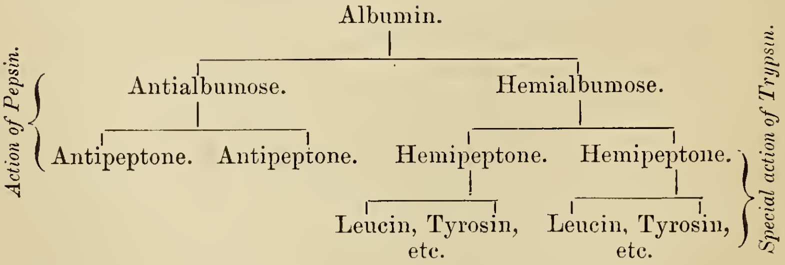

Albumin.
Antialbumate.
Hemialbumose.
Antialbumid.
Hemipeptone.
Hemipeptone.

2.

By 3—5 p. c. H_2SO_4 at 100° C.


Albumin.
Antialbumid.
Hemialbumose.
Hemipeptone.
Hemipeptone.
Leucin, Tyrosin, etc.
Leucin, Tyrosin, etc.

Decomposition of Proteids by Digestive Ferments (Enzymes).


Albumin.
Action of Pepsin.
Antialbumose.
Hemialbumose.
Antipeptone.
Antipeptone.
Hemipeptone.
Hemipeptone.
Leucin, Tyrosin, etc.
Leucin, Tyrosin, etc.
Special action of Trypsin.

The several products (antipeptone, &c.) are given in duplicate, on the hypothesis (of which there is now but little doubt) that the changes of digestion are essentially hydrolytic changes, accompanied by a deduplication; that just as a molecule of starch splits up into at least two molecules of dextrose, or as a molecule of cane-sugar splits up into a molecule of dextrose and a molecule of levulose, so a molecule of antialbumose, for instance, splits up into at least two molecules of antipeptone, and so on.

Having thus briefly stated the steps by which our present knowledge has been reached of the possible products of a digestive conversion of proteids, it now remains to deal with these products seriatim. In so doing it will be best to describe first such products as arise most largely and characteristically during the action of acids, and to treat of the albumoses and peptones subsequently.

Antialbumate. This substance is, according to Kühne, identical with Meissner's parapeptone. It is most readily formed by the fairly prolonged action of dilute acids at 40°, but it may also make its appearance, but to much smaller extent, during a peptic digestion in which but little pepsin is present. It is obtained, mixed in some cases with variable quantities of an ordinary acid albumin, by neutralising the digesting mixture, from which it is thus precipitated. As already stated, it is characterised by the property that it cannot be converted into a peptone by the most prolonged action of even the most active pepsin, while on the other hand it is readily peptonised by trypsin and yields then antipeptone, but no leucin or tyrosin. Apart from its behaviour with pepsin and trypsin, it resembles ordinary acid-albumin and syntonin in its general chemical reactions. But the latter are chemically quite distinct from antialbumate or parapeptone, for either of them may be peptonised by pepsin, and the peptones thus formed may be partly made to yield leucin and tyrosin by the subsequent action of trypsin.

Antialbumid. By the further prolonged or active treatment of antialbumate with acids it is converted into the substance to which Kühne gave the name of antialbumid. It is in all respects identical with the 'hemiprotein' of Schützenberger, and also probably with the dyspeptone of Meissner, so far as the latter was not perhaps largely composed of nucleins. It also makes its appearance, but in very small amount, during a peptic digestion, and in considerable quantity during a pancreatic. It is characterised by its relatively great insolubility in dilute acids and alkalis, so that it separates out as a granular residue during a pancreatic digestion. This residue is readily soluble in 1 p. c. caustic soda; if reprecipitated by neutralisation, it is now soluble in 1 p. c. sodium carbonate. From either of these solutions it is very completely precipitated by the addition of a little sodium chloride. In dilute alkaline solution (1 p. c. Na_2CO_3) it may be partly converted into a peptone by the action of trypsin, during which process the larger part separates out into a gelatinous coagulum or clot, which is quite unacted upon by pepsin and can only be peptonised by the prolonged action of very active trypsin in presence of a considerable amount (5 p. c.) of sodium carbonate. The peptone thus produced is antipeptone, for it yields no leucin or tyrosin by the action of trypsin.

It has been suggested above that Meissner's dyspeptone might have consisted largely of nuclein, and this possibility becomes very great in the light of the statements previously made as to the nature of casein (see p. 20) and the fact that it was during the digestion of this proteid that he obtained the so-called dyspeptone. Even as regards the similar residue left during a peptic digestion of fibrin, it has been stated that here also the dyspeptone is merely a residue (nucleins) from the cellular elements which are ordinarily entangled in the fibrin; in support of this it is stated that no dyspeptone is obtained during the digestion of fibrin prepared from filtered plasma.[1] There is however now no doubt from Kühne's researches that anti-albumid is a true proteid, not a mere undigested residue of nucleins, and that its properties are generally such as Meissner described for his dyspeptone.

The albumoses. These are the true primary products of the action of the proteolytic enzymes on proteids, and give rise by the further action of the ferments to the corresponding peptones. In accordance with Kühne's views already stated there must of necessity be at least two albumoses, antialbumose the forerunner of antipeptone, and hemialbumose of hemipeptone.

Antialbumose.[2] This substance is obtained as a neutralisation precipitate at a certain early stage of a fractionated peptic digestion of proteids. In its ordinary chemical reactions it is indistinguishable from acid-albumin or syntonin. It may be converted into a peptone by the further action of pepsin, and still more readily by the action of trypsin, so that it does not make its appearance in the final products of either a prolonged peptic or a short tryptic digestion. The peptone, into which it may be converted by either pepsin or trypsin, is antipeptone, for it cannot be made to yield any trace of leucin or tyrosin by even the most prolonged and energetic treatment with trypsin, and in this fact lies the distinction between antialbumose and either acid-albumin or syntonin. During its peptonisation by trypsin some antialbumid is simultaneously formed. Antialbumose differs from parapeptone by the fact that the latter can only be peptonised by trypsin, the former by either pepsin or trypsin.

Hemialbumose.[3] This is the best known, most characteristic and most frequently obtained by-product of proteid zymolysis.[1] It was first noticed and isolated by Meissner under the name of *a*-peptone, is identical with Bence-Jones' proteid in the urine of osteomalacia, and has also been known under the name of 'propeptone.' Of late years it has been recognised as occurring not infrequently in urine,[2] and it is more than probable that many of the older statements as to the occurrence of peptones in urine and other fluids referred really to the occurrence of hemialbumose. It is also stated to occur normally in the marrow of bones,[3] and in cerebrospinal fluid.[4] Since it is readily peptonised by trypsin with the simultaneous formation from the peptone of much leucin and tyrosin, hemialbumose scarcely makes its appearance in any appreciable quantity in the final products of a pancreatic digestion. It is best prepared by the action of a small amount of very active pepsin on a considerable mass of fibrin, previously swelled up into a gelatinous mass by the action of ·2 p.c. HCl at 40° [5]. Under the action of the pepsin the fibrin liquefies; as soon as this is complete, dilute sodium carbonate is added until the reaction is just *faintly* alkaline, by which means a bulky precipitate is obtained. This is removed by filtration and the filtrate now contains a large amount of hemialbumose and but little peptone, and may be utilised directly for the tests characteristic of the albumose.

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[1] Hammarsten, Pflüger's *Arch.* Bd. xxx. (1883), S. 440.

[2] Kühne u. Chittenden, *Zt. f. Biol.* xix. (1883), Ss. 170, 194.

[3] Schmidt-Mülheim, *antea loc. cit.* Salkowski, Virchow's *Arch.* Bd. 81. (1880), S. 552. Kühne and Chittenden, *loc. cit.* and *Zt. f. Biol.* Bd. xx. (1884), S. 11. Herth, *Monatshefte f. Chem.* Bd. v. (1884), S. 266. Straub (Dutch). See Maly's

Preparation of pure hemialbumose (Salkowski).[6] Acidulate the filtrate described above strongly with acetic acid, add an excess (37·5 grms. to each 100 c.c.) of sodium chloride, and agitate the mixture until it is saturated with salt. The hemialbumose is thus precipitated; it is now collected on a filter, washed with saturated solution of sodium chloride, dissolved again in water, and reprecipitated by acetic acid and sodium chloride. This process is repeated, and the final product is then dissolved in a minimal amount of water and freed from salt by dialysis.[7] It may then be concentrated, precipitated by alcohol and dried, first over sulphuric acid and then at 105°.

Reactions of hemialbumose. The pure dry substance is not readily soluble in distilled water, but readily soluble in *traces* of

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Bericht. Bd. XIV. (1884), S. 28. Hamburger (Dutch). See Maly's *Bericht.* Bd. XVI. (1886), S. 20.

[1] This expression may be conveniently used to denote generally the changes produced by the unorganised ferments.

[2] Salkowski u. Leube, 'Die Lehre von Harn.' 1882, Sn. 210, 350.

[3] Fleischer, Virchow's *Arch.* Bd. 81 (1880), S. 188.

[4] Halliburton, *Jl. of Physiol.* Vol. x. (1889), p. 232.

[5] For precise details see *Zt. f. Biol.* Bd. XIX. (1883), S. 184. See also Drechsel, "Anleitung zur Darstell. physiol.-chem. Präparate." Wiesbaden, 1889, S. 23.

[6] Virchow's *Arch.* Bd. LXXXI. (1880), S. 552.

[7] During the dialysis some loss of albumoses occurs, since they are slightly diffusible, but less so than the peptones. *Zt. f. Biol.*, Bd. XX. (1884) Note on p. 27.

acids, alkalis, and neutral salts (sodium chloride). These solutions give the following characteristic reactions: —

1. Acidulate fairly strongly with acetic acid and add a few drops of saturated solution of sodium chloride; a precipitate is formed which disappears on warming and comes down again on cooling. If excess of the salt is added the precipitate does not dissolve on warming.
2. Add carefully a few drops of pure nitric acid; a precipitate is formed if the acid is not in excess, which disappears on warming and comes again on cooling.
3. Add acetic acid, avoiding all excess, and then a trace of potassium ferrocyanide; a precipitate is formed which disappears on warming and reappears on cooling.
4. On the addition of caustic soda in excess and a trace of sulphate of copper the ordinary biuret reaction is obtained. This reaction distinguishes hemialbumose from other soluble proteids, with the exception of peptones.

Hemialbumose has so far been spoken of as being one uniform substance only. Kühne and Chittenden in their earlier work [1] at first distinguished merely between a soluble and insoluble form; more recently they have described four closely allied, but distinct forms of the albumose.[2] (1) *Protalbumose.* Soluble in hot and cold water and precipitable by NaCl in excess. (2) *Deuteroalbumose.* Soluble in water, not precipitated by NaCl in excess, unless an acid be added at the same time. (3) *Heteroalbumose.* Insoluble in hot or cold water; soluble in dilute or more concentrated solutions of sodium chloride, and precipitable from these by excess of the salt. (4) *Dysalbumose.* Same as heteroalbumose, except that it is insoluble in salt solutions.[3] Hemialbumose as ordinarily prepared may hence be regarded as a mixture of these several albumoses in varying proportions according to the conditions of its preparation.

The preceding statements as to the existence of four forms of hemialbumose are however contested by Herth, Straub, and Hamburger (loc. cit. on p. 42).

The peptones. Recent work has shewn that in all probability the various substances which have been described as peptones have consisted to some extent, if not largely, of a mixture of true peptones with variable quantities of albumoses. Our knowledge of the nature and properties of true peptones is at present in a state of transition, so that it is on the whole advisable to give some account of the older work as well as of the more recent.

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[1] *Zt. f. Biol.* Bd. XIX. (1883), T. 174.

[2] *Ibid.* Bd. XX. (1884), S. 11.

[3] For further details the original papers of Kühne and Chittenden must be consulted, more especially *Zt. f. Biol.* Bd. XX. (1884), S. 11. See also Neumeister, *Zt. f. Biol.* Bde. XXIII. (1887), S. 381; XXIV. (1888), S. 267; XXVI. S. 324. The preparation and separation of the albumoses is conveniently given in Röhmann's "Anleitung zum chemischen Arbeiten." Berlin, 1890, S. 48.

Preparation of peptones. For this the works of Maly,[1] Herth,[2] Henninger,[3] Kossel,[4] Hofmeister,[5] and Löw[6] should be consulted. The general properties and reactions of the peptones obtained by the above authors may be stated as follows. As precipitated by alcohol they consist of a white or yellowish powder, which is hygroscopic and extraordinarily soluble in water, and in some cases may even be deliquescent. Unless thoroughly dehydrated the powder may melt on gentle warming. From their neutral aqueous solutions they are precipitated with difficulty by a large excess of alcohol, being unchanged in the process and not becoming coagulated or insoluble by prolonged exposure to the action of the precipitant. The precipitation occurs with difficulty if at all in presence of hydrochloric acid. Peptones are not precipitated by many of the reagents which precipitate other proteids, but are precipitated by tannic acid, mercuric chloride, nitrates of mercury, and by phosphotungstic and phosphomolybdic acids in presence of hydrochloric or other mineral acids; also by the double iodides of potassium and mercury or potassium and bismuth, in presence of strong mineral acids. A very characteristic reaction is the 'biuret' or *pink* coloration which is obtained on the addition of an *excess* of caustic soda and a *mere trace* of sulphate of copper. The slightest excess of the copper salt gives a violet colour, as is the case with all other proteids, which deepens in tint on boiling. This biuret reaction is however now known to be yielded also by the albumoses (see above). Peptones are all laevorotatory and diffusible.

The diffusibility of peptones is *relatively* great in comparison with that of other forms of proteids: it is however *absolutely* small when compared with that of crystalline substances such as sodium chloride, and hence they may be separated from admixed salts by dialysis. All statements as to their absolute diffusibility, as based on earlier statements, must however be received with caution, in view of the transitional state of our information as to the properties of the true peptones.

Of late years it has been observed that the complete separation of peptones from albumoses is possible by taking advantage of the fact that the latter are all completely precipitable by saturation with *neutral* ammonium sulphate, whereas the former are not.[7] By means of this difference in the behaviour of the two classes of substances to the ammonium salt, Kühne and Chittenden have prepared what they regard as the true pure peptones as follows.[1] The products of a digestion are neutralised, filtered, very faintly acidulated with acetic acid and saturated with the ammonium salt. The filtrate from the precipitate thus obtained is largely freed from the excess of salt by careful concentration on a water-bath. The ammonium salt is then got rid of by the addition of baryta water and barium carbonate in slight excess, and after filtration these reagents are finally removed by the careful addition of dilute sulphuric acid. The peptone thus obtained may be still further purified by precipitation with phosphotungstic acid.[2] The pure peptones thus prepared are strikingly non-precipitable by many of the reagents by which other proteids may be precipitated, more especially by ferrocyanide of potassium in presence of acetic acid, a reagent by which practically all other proteids in solution are precipitated. No quantitative statements have as yet been made as to their rotatory power or diffusibility. They are stated to have such an affinity for water that a small portion of the dry substance when moistened with water exhibits the same phenomena as does phosphoric anhydride under similar conditions. They also yield an intense 'biuret' reaction with caustic soda and sulphate of copper.

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[1] Pflüger's *Arch.* Bd. IX. (1874), S. 585.
[2] *Zt. f. physiol. Chem.* Bd. I. (1877), S. 273.
[3] "De la nature et du rôle physiologique des Peptones." Paris, 1878.
[4] *Zt. f. physiol. Chem.* Bd. III. (1879), S. 58.
[5] *Ibid.* Bd. V. (1881), S. 129.
[6] Pflüger's *Arch.* Bd. XXXI. (1883), S. 408.
[7] Wenz, *Zt. f. Biol.* Bd. XXII. (1886), S. 10. Kühne, *Verhandl. d. naturhist.-med. Ver.* Heidelberg, Bd. III. (1885), S. 286.

Antipeptone may be obtained by the action of either pepsin or trypsin on antialbumose, or by the action of trypsin on antialbumate or antialbumid. When purified no leucin or tyrosin can be obtained by the most prolonged action of trypsin on this peptone.

Hemipeptone is best obtained by the action of pepsin on hemialbumose. When purified and digested with trypsin it yields much leucin and tyrosin, and in this respect alone does it differ from antipeptone.

Amphopeptone. This is the mixture of anti- and hemi-peptone resulting from the action of pepsin on proteids.

Notwithstanding the probable formation of peptones in large quantities in the stomach and intestine, to judge from the results of artificial digestion, a very small quantity only can be found in the contents of these organs.[3] They are probably absorbed as soon as formed. Another point of interest is their reconversion into other forms of proteids, since this must occur to a great extent in the body. We are however as yet ignorant of the manner in which this reverse change is effected.

It is now generally considered that the peptones are products of the hydrolytic decomposition of the proteids from which they are formed. This view is based partly upon general considerations as to the probable nature of the change, from observations of the conditions under which they are formed, and which are known to be hydrolytic in other cases, e. g. the conversion of starch into sugar by the action of enzymes and acids. There is further a certain amount of direct evidence that their formation is accompanied by the assumption of water.[1] Finally there is an increasing amount of evidence, based on analyses of proteids and the peptones which may be formed from them, that the latter contain less carbon, i. e. more hydrogen (?) and oxygen than the former.[2] But this latter evidence is as yet merely suggestive. It is however borne out by analysis of gelatin-peptones.[3] The one important fact in connection with the relationship of the peptones to the mother proteids is that they are, as already stated, products of the decomposition of the latter and of smaller molecular weight, an assumption which is warranted not only by the whole tendency of recent investigation, but more especially by the fact that whereas ordinary proteids are non-diffusible, peptones, and to a less degree the albumoses, are diffusible.

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[1] *Zt. f. Biol.* Bd. XXII. (1886), S. 423.

[2] Hirschler, *Zt. f. physiol. Chem.* Bd. XI. (1887), S. 28. Otto, *Ibid.* Bd. VIII. (1883), S. 136.

[3] Schmidt-Mülheim, *Arch. f. Physiol.* 1879, S. 39.

According to the views of some observers it is said to be possible to effect a partial reconversion of peptones into the more primary proteids from which they were obtained, by means of prolonged heating to 140—170°, and possibly by means of a dehydrating agent such as acetic anhydride.[4] But little is however definitely known as to the real nature of the products obtained by these means.

It was at one time stated that when peptones are injected into the blood-vessels, the blood speedily loses its power of clotting after removal from the body.[5] This action is now known to be due to the albumoses with which the peptones were mixed.[6] The clotting may similarly be prevented by the injection of a 1 p.c. NaCl extract of the pharynx and gullet of the leech: the cause of this has not as yet been fully worked out.[7]

During the pancreatic digestion of proteids some by-product makes its appearance which gives a characteristic violet or pink coloration on the addition of bromine, or of chlorine in the presence of acetic acid.

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[1] Danilewski, *Centralb. f. d. med. Wiss.* 1880, Nr. 42 ; 1881, Nr. 4 u. 5. *Arch. d. Sci. phys. et nat.* T. VII. (1883), p. 150, 425.

[2] Otto, *loc. cit.* Kühne u. Chittenden, *Zt. f. Biol.* XIX. 203 ; XXII. 452.

[3] Tatarinoff, *Compt. Rend.* T. 97. (1883), p. 713. Hofmeister, *Zt. f. physiol. Chem.* Bd. II. (1878), S. 299. Klug, Pflüger's *Arch.* Bd. XLVIII. (1890), S. 100 But see also Chittenden and Solley, *Jl. of Physiol.* Vol. XII. (1891), p. 33, on the gelatoses.

[4] Henninger, *loc. cit.* on p. 45. Hofmeister, *Zt. f. physiol. Chem.* Bd. II. S. 206. Pekelharing, Pflüger's *Arch.* Bd. XXII. (1880), S. 196. Kühne, *Verhand. d. naturhist.-med. Ver.*, Heidelberg, Bd. III. (1885), S. 290. Neumeister, *Zt. f. Biol.* Bd. XXIII. (1887), S. 394.

[5] Schmidt-Mülheim, *Arch. f. Physiol.* 1880, S. 33. Fano, *Ibid.* 1881, S. 277.

[6] Pollitzer, *Jl. of Physiol.* Vol. VII. (1885), p. 283.

[7] Haycraft, *Proc. Roy. Soc.* No. 231, 1884. *Arch. f. exp. Path. u. Pharm.* Bd. XVIII. (1884), S. 209. Dickinson, *Jl. of Physiol.* Vol. XI. (1890), p. 566.

The colour is not due to the peptones or albumoses (Kühne). The colouring matter obtained by the addition of these reagents has been examined by Krukenberg[1] and more recently by Stadelmann.[2]

Class VII. *Lardacein, or the so-called amyloid substance.*[3]

The substance, to which the above name is applied, is found as a pathological deposit in the spleen and liver, also in numerous other organs, such as the blood-vessels, kidneys, lungs, &c.

It is insoluble in water, dilute acids and alkalis, and neutral saline solutions.

In percentage composition it is almost identical with other proteids,[4] viz.:—

O. and S.	H.	N.	C.
24·4	7·0	15·0	53·6

The sulphur in this body exists in the oxidised state, for boiling with caustic potash gives no sulphide of the alkali. The above results of analysis would lead at once to the ranking of lardacein as a proteid, and this is strongly supported by other facts. Strong hydrochloric acid converts it into acid-albumin, and caustic alkalis into alkali-albumin. When boiled with dilute sulphuric acid it yields leucin and tyrosin;[5] by prolonged putrefaction indol, phenol, &c.[6] On the other hand, it exhibits the following marked differences from other proteids:—It wholly resists the action of ordinary digestive fluids; it is coloured red, not yellow, by iodine, and violet or pure blue by the joint action of iodine and sulphuric acid. From these last reactions it has derived one of its names, 'amyloid,' though this is evidently badly chosen; for not only does it differ from the starch group in composition, but by no means can it be made to yield sugar:[7] this latter is one of the crucial tests for a true member of the carbohydrate group. According to Heschl[8] and Cornil[9] anilin-violet (methyl-anilin) colours lardaceous tissue rosy red, but sound tissue blue.

The colours mentioned above, as being produced by iodine and sulphuric acid, are much clearer and brighter when the reagents are applied to the purified lardacein. When the reagents are applied to the crude substance in its normal position in the tissues, the colours obtained are always dark and dirty-looking.

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[1] *Verhand. d. phys.-med. Gesell.* Würzburg, Bd. xviii. (1884), Nr. 9, S. 7.

[2] *Zt. f. Biol.* Bd. xxvi. (1890), S. 491.

[3] Virchow, *Compt. Rend.* T. xxxvii. p. 492, 860.

[4] C. Schmidt, *Ann. d. Chem. u. Pharm.* Bd. cx. (1859), S. 250, and Friedreich u. Kekulé, Virchow's *Archiv*, Bd. xvi. (1859), S. 50.

[5] Modrzejewski, *Arch. f. exp. Path. u. Pharm.* Bd. i. (1873), S. 426.

[6] Weyl, *Zt. f. physiol. Chem.* Bd. i. (1877), S. 339.

[7] C. Schmidt, *loc. cit.*

[8] *Wien. med. Wochenschr.* No. 32, S. 714.

[9] *Compt. Rend.* T. lxxx. (1875), p. 1288.

Purified lardacein is readily soluble in moderately dilute ammonia, and can, by evaporation, be obtained from this solution in the form of tough, gelatinous flakes and lumps; in this form it gives feeble reactions only with iodine. If the excess of ammonia is expelled, the solution becomes neutral, and is precipitated by dilute acids.

Preparation. The gland or other tissue containing this body is cut up into small pieces, and as much as possible of the surrounding tissue removed. The pieces are then extracted several times with water and dilute alcohol, and if not thus rendered colourless are repeatedly boiled with alcohol containing hydrochloric acid. The residue after this operation is digested at 40° C., with active artificial gastric juice in excess. Everything except lardacein, and small quantities of mucin, nuclein, keratin, together with some portion of the elastic tissue, will thus be dissolved and removed.[1] From the latter impurities it may be separated by fractional decantation of the finely-powdered substance from water, alcohol, and ether.

In opposition to the older statements it has recently been stated that lardacein may be digested by pepsin in presence of hydrochloric acid.[2] The writer's own experiments lead him to believe in the results obtained by the earlier authorities.

The known products of decomposition of proteids are very numerous, varying in nature and relative amount with the conditions and reagents by means of which they are produced, and it may be similarly, though to a much less extent, with the kind of proteid employed. These products belong for the most part to well-known classes of chemical substances, and in many cases representatives of several consecutive members of any given homologous series are obtained during the decompositions.

A study of these products has not, however, up to the present time thrown any extended light upon the more minute molecular structure of the proteids, and the reason is not far to seek. It consists simply in the fact that we possess no guarantee or criterion of the purity of those proteids which can be obtained in sufficient amounts for the purposes of experiment. They may be, and probably are, mixtures of, it may be, several closely allied substances, so that the numerous products which arise during the decomposition of what is regarded in the experiment as one uniform substance, represent really the decomposition-products of *several* proteid molecules, and thus throw no light on the structure of any *one*. And the matter is still further complicated by the fact that the final products of any given decomposition do not at all necessarily represent the primary mode of breaking down of the proteid molecule; many of them may be the outcome of some secondary decomposition of the first-formed products. It may hence suffice to give a short account of the more generally important researches on the decompositions of proteids and to refer the reader for details to some larger work.[1]

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[1] Kühne and Rudneff, Virchow's *Arch.* Bd. XXXIII. (1865), S. 66.

[2] Kostjurin, *Wien. med. Jahrb.* 1886, S. 181.

The products of the decomposition of proteids by acids (HCl) have been elaborately studied by Hlasiwetz and Habermann.[2] These observers subjected proteids (casein) to the action of boiling concentrated hydrochloric acid in presence of stannous chloride for three days. From the fluid thus obtained they were able to separate out by repeated crystallisations leucin, tyrosin, glutamic and aspartic acids and ammonia; the mother liquor from the above yielded no further well-defined substances. Schützenberger,[3] treating proteids in presence of a little water with an excess of baryta in sealed tubes at 200 — 250°, observed a more profound breaking down of these substances as judged by the products of their decomposition. In addition to the products described by Hlasiwetz and Habermann he obtained small quantities of carbonic, oxalic, and acetic acids, together with other amido-acids homologous with leucin, amido-acids of other series, leuceïns,[4] glyco-protein, tyroleucin,[5] &c. The chief difference in the results obtained by the two sets of observers turns upon the non-occurrence of carbonic, oxalic, and acetic acids among the products of the action of hydrochloric acid. Drechsel[6] has however shown that if the non-crystallisable residue from Hlasiwetz and Habermann's experiments be appropriately treated with baryta in sealed tubes it readily yields carbonic acid, so that the difference may turn out after all to be more apparent than real. Interesting as are the above researches they do not as yet enable us to form any clear idea of the probable molecular composition of proteids. According to Schützenberger the relative amounts of carbonic acid and ammonia which make their appearance are the same as would have arisen from a similar treatment of urea with caustic baryta, and from this and the fact of the preponderating appearance of amido-acids by the action of the alkaline oxide, he regards the proteids as complex ureides: that is to say, as combinations of urea with amido-acids belonging to several series such as the leucic and aspartic.[1] In support of this view the work of Grimaux[2] may be mentioned. By fusing together aspartic anhydride and urea he obtained a substance resembling a proteid in several of its reactions, and yielding aspartic acid, carbonic acid and ammonia by treatment with baryta. It has not however as yet been shown that this substance can be made to yield urea, and further, no one has ever succeeded in obtaining urea as a direct product of the decomposition of a proteid. Further, as against the view of the ureide nature of proteids, Löw's views as to the probable non-existence of amido-acid residues in the proteid molecule must not be lost sight of.[3]

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[1] Ladenburg's *Handwörterbuch d. Chem.* Bd. III. S. 541. Beilstein's *Hdbch. d. Chem.* Bd. III. S. 1258.

[2] *Anzeig. d. Wien. Akad.* 1872, S. 114; 1873, Nr. 15. *Ann. d. Chem. u. Pharm.* Bd. 159 (1871), S. 304, Bd. 169 (1873), S. 150. *Jn. f. prakt. Chem.* (2) Bd. VII. S. 397. See also E. Schulze, *Zt. f. physiol. Chem.* Bd. IX. (1885), Sn. 63, 253.

[3] *Ann. de Chim. et de Phys.* (5 Sér.) T. XVI. (1879), p. 289. *Bull. de la Soc. Chim.* XXIII. 161, 193, 216, 242, 385, 433; XXIV. 2, 145; XXV. 147. Also in *Chem. Centralb.* 1875, Sn. 614, 631, 648, 681, 696; 1876, S. 280; 1877, S. 181. *Compt. Rend.* T. 101, (1886), p. 1267. See also Nasse, Pflüger's *Arch.* Bde. VI. (1872), 589; VII. 139; VIII. 381.

[4] *Compt. Rend.* T. 84 (1877), p. 124.

[5] *Ibid.* T. 106 (1888), S. 1407.

[6] *Jn. f. prakt. Chem.* (N. F.) Bd. XXXIX. (1889), S. 425.

The older statements of Béchamp[4] and Ritter[5] as to the formation of urea from proteids by the action of potassium permanganate are erroneous.[6] The most recent refutation of their views is due to Lossen,[7] who finds that traces of guanidin may make their appearance but no urea. This substance might however be easily mistaken for urea since its compounds with oxalic and nitric acids closely resemble those of urea with the same acids. Although guanidin when boiled with sulphuric acid or baryta water readily yields urea (and simultaneously ammonia) this can in no way be taken as implying a possible formation of urea from proteids directly. Quite recently a crystalline base called 'lysatin,' which readily yields urea when boiled with baryta water,[8] has been isolated from among the products of the decomposition of casein by hydrochloric acid and chloride of zinc. The formula of this base is given as $C_6H_{11}N_3O$, thus placing it in close compositional relationship with kreatin $C_4H_9N_3O_2$ and kreatinin $C_4H_7N_3O$.

It cannot as yet be said that we possess any real knowledge of the constitution of proteids, and the question will probably remain unsolved until some entirely new departure is made in attacking the problem, or until some new property of proteids is discovered by which their absolute purity may be determined as the necessary preliminary to the whole investigation. The so-called crystallised proteids (see above, p. 6) have not as yet been prepared in sufficient quantity[1] to admit of the easy and decisive application of the modern methods of organic chemistry to the elucidation of their molecular structure. Work in this direction on a really large scale could scarcely fail to yield important results. Schrötter[2] has recently described the preparation of benzoylated ethers of the albumoses, and intends to apply the method to other proteids and to study the products of decomposition and oxidation of these substances. Whether any real advance will be made in this direction cannot be foretold, but this new departure is of considerable prospective importance.

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[1] For Schützenberger's most recent attempts to synthetise proteids, see *Compt. Rend.* T. 112 (1891), p. 198.

[2] *Gaz. méd.* 1879, p. 521. *Compt. Rend.* T. 93 (1881), p. 771.

[3] *Jn. f. prakt. Chem.* Bd. XXXI. (1885), S. 129.

[4] *Ann. d. Chem. u. Pharm.* Bd. C. (1856), S. 247. *Compt. Rend.* T. LXX., p. 866. T. LXXIII., p. 1323.

[5] *Ibid.* T. LXXIII., p. 1219.

[6] See Städeler, *Jn. f. prakt. Chem.* Bd. LXXII. (1857), S. 251. Löw, *Ibid.* (N. F.) Bd. III. (1871), S. 180. Tappeiner, *Ber. k. Sächs. Gesell.* 1871.

[7] *Ann. d. Chem. u. Pharm.* Bd. 201 (1880), S. 369.

[8] Drechsel, *Ber. d. d. Chem. Gesell.* Jahrg. XXIII. (1890), S. 3096. Cf. Siegfried, *Ibid.* Jahrg. XXIV. S. 418.

No account of the constitution of proteids would be complete without a reference to the views and theories of Pflüger, and of Löw and Bokorny. Pflüger[3] starting from the characteristic differences between the products obtained by decomposing dead proteids by chemical means out of the body, and the products which arise by the natural decomposition (metabolism) of living proteids (protoplasm) in the body, has put forward a view as to the difference of living and dead proteid. He considers that in dead proteid the nitrogen exists in the amide form, while in living proteid it is present in the less stable cyanic form. The building-up of living proteid from dead he regards as being carried on by the ether-like union of the isomeric living and dead proteid molecules, accompanied by the elimination of water. During this process the nitrogen of the dead proteid passes into the cyanic condition, and if this is repeated and accompanied by polymerisation the formation of a large and unstable living proteid molecule may be readily accounted for. He further draws attention to the readiness with which polymerisation occurs in the cyanic series and the extraordinarily high molecular energy of cyanogen. Löw and Bokorny[4] deal also with the probable mode by which, in the case at least of plant cells, the complex proteid molecule may be built up out of the simpler substances from which these obtain their nitrogen. They consider there is evidence of the existence in living plant cells of some substance of an aldehyde nature. Starting with formic aldehyde, by its union with ammonia the aldehyde of aspartic acid might be obtained, and by polymerisation of the latter in presence of sulphur and with the exit of water a substance with the same composition as an ordinary proteid would arise. Their speculations are ingenious, but it cannot by any means be said that their views are established. Asparagin, from which aspartic acid is readily obtained, undoubtedly plays an all-important part in the constructive nitrogenous metabolism of plants; but as yet the aldehyde of aspartic acid has not been prepared by any chemical means, and Baumann[1] has cast great doubt on the reliability of the methods by which the above authors have endeavoured to prove the existence of aldehydes in the protoplasm of the living plant cells. And it is probably significant that the reactions by which the presence of the aldehydes is supposed to be shown are only well marked in the case of the cells of the lowest plants; in the case of animal cells they are more usually wanting.

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[1] But see Chittenden and Hartwell *Jl. of Physiol.* Vol. xi. (1890), p. 435.

[2] *Ber. d. deutsch. chem. Gesell.* Jahrg. XXII. (1889), S. 1950.

[3] Pflüger's *Arch.* Bd. x. (1875), S. 332.

[4] Löw and Bokorny's work may be most conveniently quoted by reference to the following volumes of Maly's *Jahresbericht d. Thierchem.* Bde. x. (1880), S. 3; XI. 391, 394; XII. 380; XIII. 1; XIV. 349, 474; XVI. 8; XVII. (1887), 395. See also *Biol. Centralb.* Bd. I. (1881), S. 193; VIII. (1888), S. 1.

THE ENZYMES OR SOLUBLE UNORGANIZED FERMENTS.[2]

Chemists have for a long time been familiar with an extensive, and still increasing class of reactions which occur solely, or in some cases most readily, in presence of minute quantities of some substance which does not itself appear to enter directly into the reaction; in other words the causative agent is found to have itself undergone no obvious change during the reactions which it has set up between the other substances. Striking instances of such reactions are observed in the preparation of ether from alcohol by means of sulphuric acid and in the manufacture of sulphuric acid itself. In the former case a small quantity of sulphuric acid is theoretically able to convert an indefinitely large quantity of alcohol into ether, and in practice the limit is determined simply by the occurrence of secondary decompositions between the reagents. Similarly during the manufacture of sulphuric acid a minute quantity of nitric oxide suffices in the presence of water to convert an indefinitely large amount of sulphurous anhydride into sulphuric acid. Of late years a large number of reactions have been found to depend for their occurrence upon the presence of the minutest traces of water; thus *dry* chlorine has no action on dry sodium, and dry hydrochloric acid gas and oxygen do not react even when exposed to bright sunlight, neither do *dry* oxygen and carbonic oxide explode on the passage of an electric spark. The fact of immediate interest in each of the above instances is that a minute trace of the substance which determines the occurrence of the reaction is able to produce change in an indefinitely large mass of the other reagents without itself undergoing any final alteration. Turning to the chemistry of animal and vegetable cells it is found that in many cases substances may be extracted from them which possess to an even more striking degree the property of inducing change in an indefinitely large mass of certain other substances without themselves undergoing any observable alteration. These agents are known as the enzymes or soluble ferments, and the essential conception of an enzyme is summed up in the above statement of the most remarkable characteristic of their activity. Further investigation of these enzymes shows that their activity is dependent upon many subsidiary factors which are more or less common to them all. Thus their activity is largely dependent upon temperature, being absent at sufficiently low temperatures, increasing as the temperature is raised to a certain optimal point which varies slightly for different enzymes, then again diminishing as the temperature is further raised, and finally disappearing. By the action of a sufficiently high temperature they permanently lose their characteristic powers and are now spoken of as being 'killed.' Again the enzymes are extremely sensitive to the reaction, whether acid, alkaline, or neutral, of the solutions in which they are working, also to the presence or absence of various salts, some of which merely inhibit their action while others permanently destroy it; and their activity is in all cases lessened and finally stopped by the presence of an excess of the products to whose formation they have given rise. It has been already said that an enzyme may be killed by exposure to a high temperature, but this only holds good when they are in solution, or if in the solid form they are heated in a moist condition. When *perfectly dry* they may be heated to 100° —160° without any permanent loss of their powers.[1] It will be seen that so far the enzymes have been characterised solely with reference to the peculiarity of their mode of action and to the influence of surrounding conditions upon that activity, and the question of their probable chemical composition has been left untouched. Notwithstanding the frequent endeavours which have been made to prepare the enzymes in a pure condition, it is unwise to lay any great stress upon the results of the analysis of these so-called 'pure ferments,' bearing in mind that, as in the case of the proteids, no criterion of their purity exists. This much however may be said. In the majority of cases, analysis shows that their composition approximates more nearly to that of a proteid than of any other class of substances, and this is apparently true even when they do not yield to any marked degree the reactions (xanthoproteic, &c.) which are characteristic of a true proteid. Ordinarily it is almost impossible to obtain an enzyme solution of any considerable activity which is free from proteid reactions, and hence many authors are

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[1] Pflüger's *Arch.* Bd. XXIX. (1882), S. 400. See also Hoppe-Seyler, *Zt. f. physiol. Chem.* Bd. X. (1886), S. 39.

[2] It appears advisable to use the term 'enzyme' (Kühne, *Unters. a. d. physiol. Inst. Heidelb.* Bd. I. 1878, S. 293) to denote the soluble unorganised ferments generally, reserving the older name of 'ferment' for the organized agents such as yeast to which it was first applied. If this be done it will be convenient to use the expression 'zymolysis' to denote the changes produced by the enzymes in their action on other substances, and to apply the term 'fermentation' to the action of the organised ferments. In this way 'zymolysis' corresponds to the German 'Ferment-wirkung,' and 'fermentation' to 'Gährung.'

[1] Hüfner, *Jn. f. prakt. Chem.* Bd. v. (1872), S. 372. Al. Schmidt, *Centralb. f. d. med. Wiss.* 1876, S. 510. Salkowski, Virchow's *Arch.* Bd. LXX. (1876), S. 158; LXXXI. (1880), S. 552. Hüppe, *Mittheil. d. Kaiserl. Gesundheitsamtes*, I. 1881.

inclined to regard these bodies as being really of proteid nature. But this is a point which is as yet by no means settled, as the following considerations show. The sole means at our disposal of determining the presence of an enzyme is that of ascertaining the change which it is able to bring about in other substances, and since the activity of the enzymes is extraordinarily great, a minute trace suffices to produce a most marked effect. From this it follows that the purified enzymes which give distinct proteid reactions might merely consist of very small quantities of a true non-proteid enzyme adherent to or mixed with a residue of inert proteid material. Again on the other hand it is similarly possible that the purified enzymes which have been described as devoid of proteid reaction really consist of some inert non-proteid material with which a trace of what is really a true proteid enzyme is admixed, the amount of enzyme being too small to yield any of the reactions characteristic of proteids. The occurrence or absence of proteid reactions in a solution of an enzyme cannot therefore settle the nature of the enzyme, and for similar reasons a mere analysis of the separated enzyme is also inconclusive ; the balance of recent opinion appears to be in favour of the view that the enzymes are proteid in nature, but this is still an open question.

Many of the purified enzymes have been analyzed and the results show in many cases a percentage of carbon considerably lower than that of a true proteid. Kühne's purest trypsin had the following percentage composition: C = 47·22 — 48·09 ; H = 7·15 — 7.44 ; N = 12·59 — 13·41 ; S = 1·73 — 1·86. For other analyses see Aug. Schmidt.[1] Hüfner,[2] Barth.[3] But see also Wurtz[4] and Löw.[5]

The enzymes are possessed of certain properties, more or less common to them all, by means of which they may be separated from the tissues in which they primarily occur, and isolated from the solutions thus obtained. Soluble in water, they may be precipitated unchanged from this solution by the addition of an excess of absolute alcohol. They may also in many cases be precipitated from their aqueous or other solution by saturation with neutral ammonium sulphate.[6] They are conveniently soluble in glycerine[7] from which they may as before be precipitated by an excess of alcohol. None of the enzymes are diffusible and hence they may readily be freed from any admixed diffusible substances by means of dialysis.[1] They possess further the remarkable property of adhering with great tenacity to any finely divided precipitate which is formed in the solutions in which they are present, more particularly if the precipitate is of a viscid or gelatinous nature.[2] It is not however possible to base upon the above properties any general method of preparing the enzymes which is equally applicable to each of them; some are most readily prepared in a fairly pure state by one method, some by another, and very many by the conjoined application of two methods. A further consideration must not be lost sight of in connection with the separation of the enzymes from the parent tissues; this is the fact that in some cases the enzymes do not exist in the free and active conditions in the cells of the respective tissues, but in the form of an inactive antecedent, to which the name of 'zymogen' is usually applied.[3] Hence to obtain an active extract it is frequently necessary to treat the tissue with some such reagent as shall ensure the conversion of the zymogen into the active enzyme.

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[1] *Inaug. Diss.* Tubingen, 1871.

[2] *Jn. f. prakt. Chem.* N. F. Bd. v. (1872), S. 372.

[3] *Ber. d. deutsch. Chem. Gesell.* Jahrs. xi. (1878), S. 474.

[4] *Compt. Rend.* T. xc. (1880), p. 1379 ; xci. p. 787.

[5] Pflüger's *Arch.* Bd. xxvii. (1882), S. 203.

[6] Kühne, *Verhand. d. naturh.-med. Ver.* Heidelb. iii. 1886, S. 463. Also *Centralb. f. d. med. Wiss.* 1886, Nr. 45. Krawkow (Russian). See *Ber. d. deutsch. chem. Gesell. Referatband.* 1887, S. 735 or Maly's *Jahresber.* xvii. S. 466.

[7] v. Wittich, Pflüger's *Arch.* Bd. ii. (1869), S. 193.

During prolonged digestions it is essential to insure the absence of any changes due to the development of bacteria or other organisms. The most suitable antiseptics for this purpose are salicylic acid (·1 p.c.) and thymol (·5 p. c.). These reagents are dissolved in a small quantity of alcohol and added in the above proportions to the digestive mixture.

It is frequently a matter of the utmost importance to determine whether the hydrolytic power of any given preparation is due to the action of a soluble enzyme or of a ferment (organised). The discrimination is most readily effected by carrying on the digestion in presence of chloroform, which is inert towards the enzymes but inhibits the activity of ferment organisms.[4]

SPECIAL DESCRIPTION OF THE MORE IMPORTANT ENZYMES.[5]

Ptyalin.

While occurring chiefly and characteristically in saliva, a similar enzyme may be obtained in minute amount, but fairly constantly, from almost any tissue or fluid of the body, more particularly in the case of the pig. It was first separated out from saliva, but in an impure condition, by Mialhe, who precipitated the saliva with an excess of absolute alcohol.[6] It has been prepared in the purest (?) form by Cohnheim.[1] His method consists in the addition of phosphoric acid to the saliva until it is strongly acid; the mixture is then neutralised by the careful addition of lime-water, whereupon a copious precipitate of phosphate of lime is formed. This carries down with it a large proportion of the proteids which are present, together with all the ptyalin. On extraction of the precipitate with a volume of water equal to that of the saliva originally employed, the enzyme passes chiefly into solution, since it is less firmly adherent to the precipitate than are the proteids; it may now be purified still further by repeating the above process and finally precipitating with absolute alcohol. Prepared in this way, the enzyme is obtained as a fine white amorphous powder. Dissolved in water it is extremely active in hydrolysing starch, and the solution yields none of the reactions most typically characteristic of proteids. On these grounds it is asserted that ptyalin is not a proteid, but the evidence is not conclusive. More recently this enzyme has been prepared as follows.[2] Saliva is diluted with an equal volume of water, and saturated with neutral ammonium sulphate. The precipitate thus formed is treated on the filter for five minutes with strong alcohol, removed from the filter, and further treated with absolute alcohol for one or two days. It is now dried at 30°, and yields, on extraction with a volume of water equal to that of the original saliva, a solution which is actively zymolytic, and is stated to be free from all proteid reactions. The hydrolytic activity of ptyalin is most marked in neutral or nearly neutral solutions.[3]

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[1] Maly, Pflüger's *Arch.* Bd. IX. (1874), S. 592.

[2] Brücke, *Sitzb. d. Wien. Akad.* Bd. XLIII. (1861), S. 601. Danilewsky, Virchow's *Arch.* Bd. XXV. (1862), S. 279. Cohnheim, Virchow's *Arch.* Bd. XXVIII. (1863), S. 241.

[3] Heidenhain, Pflüger's *Arch.* Bd. X. (1875), S. 583.

[4] Müntz, *Compt. Rend.* T. LXXX. (1875), p. 1255.

[5] Consult the article 'Fermente' by Emmerling in Ladenburg's *Handwörterbuch d. Chem.* Bd. IV. 1887, S. 95.

[6] *Compt. Rend.* T. XX. (1845), pp. 954, 1485.

An amylolytic enzyme is found in urine.[4]

No experiments have as yet established the existence of any zymogen of ptyalin (ptyalinogen).[5]

The amylolytic enzyme of the pancreas.

The secretion of the pancreas is even more active than saliva in effecting the hydrolysis of starch.[6] This property is dependent upon the presence in this secretion of an enzyme which in many ways closely resembles ptyalin, but differs from it markedly in its greater power of effecting a more complete decomposition of the starch than can ptyalin. Under ordinary conditions the only sugar formed by the action of ptyalin on starch is maltose; if, however, the action is prolonged, small amounts of dextrose may, it is stated, also make their appearance as the result of the further action of the enzyme on the first-formed maltose.[1] But this is by no means quite certainly the case, and without doubt no dextrose is obtained during a digestion of moderate duration. The pancreatic enzyme, on the other hand, not only rapidly converts starch into maltose, but further converts this maltose into dextrose in considerable quantity during a digestion of relatively short duration in comparison with that required for its production by the action of ptyalin.[2] The secretion of the pancreas is of extremely complicated composition, and contains in addition to the amylolytic at least two other well characterised enzymes; from these the former has as yet been only very imperfectly separated, so that scarcely anything is known of its chemical nature as distinct from its converting powers. According to von Wittich the amylolytic enzyme is separable from the others by treating the gland with ether and alcohol before its extraction with glycerine, to which reagent it then yields only the amylolytic enzyme;[3] Hüfner, however, obtained a mixture of enzymes by von Wittich's method.[4] Experiments on the separation of the enzymes have also been made by Danilewsky[5] and Paschutin;[6] but the most successful outcome of any method which may be employed simply results in the production of an extract which is preponderatingly amylolytic, but is by no means free from the other enzymes. An active amylolytic extract is best prepared by Roberts' method,[7] in which the finely minced pancreas is extracted for five or six days with four times its weight of 25 p.c. alcohol, the mixture being frequently stirred. The pancreas of the pig yields the most certainly active extracts, and more particularly if the gland is kept for 24 hours after removal from the body, and is then treated for a few hours with dilute (·5 p.c.) acetic acid before its final extraction with alcohol.

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[1] Virchow's *Arch.* Bd. XXVIII. (1863), S. 241.

[2] Krawkow, *loc. cit.*

[3] Langley and Eves, *Jl. of Physiol.* Vol. IV. (1882), p. 18.

[4] For litt. see ref. 1, sub Pepsin, p. 61.

[5] Langley, *Jl. of Physiol.* Vol. III. (1881), p. 288.

[6] Kühne, *Lehrb. d. physiol. Chem.* 1868, S. 117. Maly in Hermann's *Hdbch. d. Physiol.* Bd. V. 2, S. 194.

Benger's 'liquor pancreaticus' is, when freshly prepared, possessed of extraordinarily active amylolytic powers. From it an extremely pure and active solution of the enzyme may be obtained by adding to it four times its volume of strong alcohol and filtering off the precipitate thus formed; the precipitate is then rapidly washed with alcohol, dried in the air, and dissolved in water.

The secretion and extracts of the small intestine possess to a slight extent the power of slowly hydrolysing starch into maltose; the conversion being more rapid if portions of the mucous membrane of the intestine be finely divided and immersed in the starch solution.[1] The tissue and its extracts, on the other hand, possess to a very marked extent the power of rapidly effecting a conversion of maltose into dextrose; this is of great physiological significance, inasmuch as it points to the probability that the carbohydrates are absorbed from the intestine as dextrose and not as maltose, — a view which is supported by the fact that maltose does not appear to be capable of direct assimilation, but is excreted largely unchanged if injected into the blood.[2] If this be so, then it is as dextrose that the liver receives its supply of carbohydrate material for the formation of glycogen, — a fact which is of no small interest when we know that the liver discharges the carbohydrate which results from the reconversion of glycogen into sugar as dextrose.[3] (See also sub glycogen.)

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[1] Musculus und Gruber, *Zt. f. physiol. Chem.* Bd. II. (1878), S. 177. Musculus und v. Mering, *Ibid.* S. 403. v. Mering, *Ibid.* Bd. V. (1881), S. 185.

[2] Brown and Heron, Liebig's *Ann.* Bd. CXCIX. (1879), S. 165. *Ibid.* Bd. CCIV. (1880), S. 228. *Proc. Roy. Soc.* No. 204 (1880), p. 393. Confirmed also by the author's own experiments.

[3] Pflüger's *Arch.* Bd. II. (1869), S 198.

[4] Hüfner, *Jn. f. prakt. Chem.* N. F. Bd. V. (1872), S. 372.

[5] Virchow's *Arch.* Bd. XXV. (1862), S. 279. But see Lossnitzer, *Diss.* Leipzig, 1864.

[6] *Arch. f. Anat. u. Physiol.* Jahrg. 1873, S. 382.

[7] *Proc. Roy. Soc.* Vol. XXXII. (1881), p. 145. See also *Digestion and Diet*, 1891, pp. 16, 69.

Cane-sugar has been shown by Bernard to be similarly incapable of assimilation; if injected into the blood it is excreted in the urine unchanged. When taken through the alimentary canal it is probably inverted or converted into a mixture of dextrose and lævulose, which are then assimilable.

The conversion of hepatic glycogen into sugar as a preliminary to its discharge from the liver has more usually been regarded as dependent upon the activity of some special hepatic enzyme. This view is now no longer tenable in face of the negative evidence as to its existence obtained by more recent observers.[4] (See also sub glycogen.)

Pepsin.

This is the characteristic proteolytic enzyme of gastric juice. It was first separated out in an approximately pure form by Brücke.[5]

His method was as follows. The mucous membrane of the stomach is separated from the muscular coats, finely chopped and digested with a large volume of 5 p. c. phosphoric acid. The fluid thus obtained is strained off through linen, and filtered, and lime-water is added until the reaction is just not quite neutral; by this means a precipitate of calcium phosphate is obtained to which all the pepsin is adherent. The precipitate is now filtered off, dissolved in a minimal amount of dilute hydrochloric acid and again precipitated by the addition of lime-water; this second precipitation frees the pepsin largely from the proteids which were at first carried down with it. This second precipitate is now as before dissolved in dilute hydrochloric acid. From this the pepsin is separated as follows. Cholesterin is dissolved in a mixture of four parts of alcohol and one of ether, and this solution is introduced *below* the solution of pepsin by means of a long thistle-tube. As soon as the cholesterin comes in contact with the water it separates out and the separation is completed, as a finely granular mass, by violently shaking the vessel in which the mixture is contained. The pepsin adheres now to the cholesterin, which is filtered off, washed first with water faintly acidulated with acetic acid and finally with pure water. On treating the mass with pure ether in a separating-funnel the cholesterin goes into solution in the ether which forms an upper layer, below which is an aqueous solution of pepsin, which must be shaken up several times with renewed portions of ether until all the cholesterin has been extracted. The aqueous solution of the enzyme thus obtained is exposed to the air until it is free from ether, and is then filtered. It may be further purified by dialysis, and is now found to give none of the reactions characteristic of proteids, and to be precipitable only by the acetates of lead. It yielded no trace of opalescence on the addition of tannic acid, though this is capable of detecting one part of proteid in 100,000 of solvent.[1]

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[1] Brown and Heron, *Proc. Roy. Soc.* No. 204 (1880), p. 393. Liebig's *Ann.* Bd. CCIV. (1880), S. 228. Vella, Moleschott's *Untersuch. zu Naturlehre*, Bd. XIII. (1881), S. 40. Bourquelot, *Compt. Rend.* T. XCVII. (1883), p. 1000.

[2] Bimmermann, Pflüger's *Arch.* Bd. XX. (1879), S. 201. Philips (Dutch Diss.). See Maly's *Bericht*, Bd. XI. (1881), S. 60. Dastre et Bourquelot, *Compt. Rend.* T. XCVIII. (1884), p. 1604. Bourquelot, *Jn. de l'Anat. et de la Physiol.* T. XXII. (1886), p. 161.

[3] Nasse, Pflüger's *Arch.* Bd. XIV. (1877), S. 479. Seegen, *Ibid.* XIX. (1879), S. 123. Seegen und Kratschmer, *Ibid.* XXII. (1880), S. 206. Külz, *Ibid.* XXIV. S. 52. Musculus und v. Mering, *Zt. f. physiol. Chem.* Bd. II. (1878), S. 417.

[4] Eves, *Jl. of Physiol.* Vol. V. (1884), p. 342. (Gives litt. to date.) Dastre, *Arch. de Physiol.* (4) T. I. (1888), p. 69.

[5] *Sitzb. d. Wien. Akad.* Bd. XLIII. (1861), S. 601. See also his *Vorles. über Physiol.* (sub pepsin).

From the reactions of the pepsin solution obtained by Brücke's method, it seems justifiable to consider that the enzyme is not really a proteid. The same conclusion may be deduced from the more recent investigation of Sundberg.[2] No analyses of purified pepsin appear to have been made as yet, so that the views as to its non-proteid nature are based solely upon the reactions of its solutions as described by Brücke and Sundberg, reactions which, as already pointed out, are not really conclusive.

Preparation of peptic digestive fluids. If a few drops of a glycerine extract of gastric mucous membrane be added to dilute (·2 p. c.) hydrochloric acid, or if the tissue be simply extracted for a short time with the dilute acid and the extract be filtered, a solution is obtained which suffices for demonstration and ordinary purposes.[3] When however a peptic extract is required for research purposes it is essential to adopt some more elaborate method which yields a product as free as possible from admixed substances; one of the best is that of Maly.[4] The mucous membrane is digested, as in Brücke's method, with phosphoric acid and the fluid precipitated with lime-water. The precipitate of calcium phosphate is then filtered off, washed, and dissolved in dilute hydrochloric acid, and this solution is then dialysed until it is free from chlorine and phosphates, and on acidulating with hydrochloric acid is ready for use.

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[1] Hofmeister, *Zt. f. physiol. Chem.* Bd. II. (1878), S. 292.

[2] *Zt. f. physiol. Chem.* Bd. IX. (1885), S. 319. But see Löw, Pflüger's *Arch.* Bd. XXXVI. (1885), S. 170.

[3] See also Kühne and Chittenden, *Zt. f. Biol.* Bd. XIX. (1883), S. 184.

[4] Pflüger's *Arch.* Bd. IX. (1874), S. 592.

Owing to the relatively slow diffusibility of albumoses and peptones, mere dialysis of a solution of pepsin in which these substances are present does not, within any reasonable time, suffice to yield an even comparatively pure solution of the enzyme.

Many forms of commercially prepared pepsin are obtained by digesting the gastric mucous membrane with dilute hydrochloric acid; the solution thus obtained is then saturated with some salt such as NaCl, $MgSO_4$ or $CaCl_2$, whereupon a scum rises to the surface, consisting chiefly of proteid matter to which the pepsin is adherent. This scum is then removed, frequently mixed with milk-sugar and dried at a low temperature.[1]

Pepsin does not exist preformed in the cells of the gastric glands, but as a zymogen to which the name of pepsinogen is given; this is readily converted into pepsin by the action of hydrochloric acid.[2]

Unlike ptyalin the hydrolytic activity of pepsin is manifested only in presence of an acid. The most efficient acid in this respect for artificial digestions is hydrochloric of a strength of ·2 p. c.[3] The average percentage of this acid may be stated as ·2 p. c. in normal gastric juice, but it varies slightly in the case of different animals.[4] Other acids may be substituted for the hydrochloric, the optimal percentage varying for the several acids.[5]

A remarkable peptonising enzyme (papaïn), exits in the milky juice of an East and West Indian plant, Carica Papaya. Any description of this enzyme and its properties lies outside the scope of this work; all necessary information may be obtained by referring to the papers quoted below.[6]

Traces of pepsin and other enzymes are frequently found in urine; the literature of the subject up to the present date is fully quoted in the papers to which a reference is here given.[7]

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[1] Scheffer. See abstract in Maly's *Jahresbericht.* Bd. III. (1873), S. 159. Selldén (Swedish), *Ibid.* S. 159.

[2] Ebstein und Grützner, Pflüger's *Arch.* Bd. VIII. (1874), S. 122. Langley, *Jl. of Physiol.* Vol. III. (1881), p. 278. Langley and Edkins, *Ibid.* Vol. VII. (1886), p. 371. Podwyssozky, Pflüger's *Arch.* Bd. XXXIX. (1886), S. 62.

[3] Ad. Mayer, *Zt. f. Biol.* Bd. XVII. (1881), S. 356.

[4] Bidder und Schmidt, *Die Verdauungssäfte,* Leipzig, 1852, S. 46. Heidenhain, Pflüger's *Arch.* Bd. XIX. (1879), S. 152.

[5] Davidson und Dieterich, *Arch. f. Anat. u. Physiol.* Jahrg. 1860, S. 688. Petit. See ref. in Maly's *Bericht.* Bd. X. 1880, S. 308. Also Ad. Mayer, *loc. cit.*

[6] Wurtz et Bouchut, *Compt. Rend.* T. LXXXIX. (1879), p. 425. Wurtz, *Ibid.* T. XC. p. 1379; T. XCI. p. 787. Polak (Dutch). See Abst. in Maly's *Jahresber.* 1882, S. 254. Martin, *Jl. of Physiol.* Vol. V. (1883), p. 213; VI. p. 336.

[7] Stadelmann, *Zt. f. Biol.* Bd. XXIV. (1888), S. 226. See also Wasilewski (Russian). Abst. in Maly's *Bericht.* (1887), S. 193. H. Hoffmann, Pflüger's *Arch.* Bd. XLI. (1887), S. 148. Helwes, *Ibid.* Bd. XLIII. (1888), S. 384.

Trypsin.

The proteolytic enzyme of pancreatic juice. This appears to have been first separated from the other enzymes which exist in pancreatic juice by Danilewsky.[1] More recently Kühne has prepared it in quantity and in what must be presumed to be a pure (?) form, by an elaborate and lengthy process, for the details of which his original work must be consulted.[2] The composition of the enzyme as prepared by Kühne was found to be remarkably complex, as shown by the fact that when dissolved in water and boiled it is split up with the formation of 20 p. c. coagulated proteid and 80 p. c. albumose. It might at first sight appear probable from this that the purified enzyme was in reality a mixture of the true enzyme with other substances (proteid) to whose decomposition on boiling the coagulated proteid and albumose were due, and some authors have taken this view.[3] This seems however to be negatived by the fact that Kühne digested his trypsin for several weeks in dilute alkaline solution and did not observe the formation of the least trace of peptone, leucin, or tyrosin. The percentage composition of the enzyme has been quoted on p. 55, from which it appears to contain distinctly less carbon than a true proteid.

Preparation of solutions of trypsin for digestion experiments. The following method due to Kühne yields an extraordinarily pure and active tryptic solution; unfortunately it is a somewhat lengthy process.[4]

One part by weight of pancreas which has been extracted with alcohol and ether is digested at 40° for 4 hours with 5 parts of ·1 p. c. salicylic acid. The residue after being squeezed out is further digested for 12 hours with 5 parts of ·25 p. c. Na_2CO_3, and the residue is again squeezed out. The acid and alkaline extracts are now mixed together, the whole made up to ·25 — ·5 p. c. Na_2CO_3, and digested for at least a week in presence of ·5 p. c. thymol. By this means all the first formed albumoses are fully converted into peptones; this is essential. At the end of the week the fluid is allowed to stand in the cold for 24 hours, filtered, faintly acidulated with acetic acid, and saturated with *neutral* ammonium sulphate. By this means all the trypsin is separated out and may be collected on a filter, where it is washed with the ammonium salt (sat. sol.) till free from peptones. It is now finally dissolved off the filter in a little ·25 p. c. solution of Na_2CO_3, to which thymol is added and thus an extremely active and very pure digestive solution is obtained. Ten grams of the original pancreas yield 80 — 100 c. c. of extract.

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[1] Virchow's *Arch.* Bd. xxv. (1862), S. 279.

[2] *Verhandl. d. naturhist.-med. Ver. Heidelbg.* (N.F.), Bd. I. (1876), S. 194.

[3] Löw, Pflüger's *Arch.* Bd. xxvii. (1882), S. 209.

[4] *Verhand. d. naturhist.-med. Ver. Heidelbg.* (N.F.), Bd III. (1886), S. 463. Also *Centralb. f. d. med. Wiss.* 1886, Nr. 45.

Although Benger's 'liquor pancreaticus' contains in addition to the enzymes both leucin and tyrosin together with proteids, it is so actively proteolytic that the small amount required to yield an active digestive solution introduces an amount of impurities which may be neglected in many cases. The above impurities may be largely got rid of by precipitating out the enzymes with alcohol as described on p. 58.

Although trypsin exhibits its hydrolytic powers to the greatest advantage in presence of an alkali, its activity is scarcely so directly related to the alkali as is that of pepsin to dilute hydrochloric acid. Thus it will digest proteids, although much more slowly in a neutral solution and even in presence of dilute (·012 p. c.) hydrochloric acid, but the slightest excess (·1 p. c.) of the acid destroys it.[1] In connection with these statements it must however be borne in mind that proteids have the power of readily combining with acids, hence the addition of say ·1 p. c. of hydrochloric acid to a digestive mixture does not imply that there is then ·1 p. c. of *free* acid in the solution.[2]

This comparative independence of tryptic activity in its relations to the reaction of the digestive mixture is doubtless of considerable physiological significance. The reaction of the contents of the small intestine is very variable. The chyme as discharged from the stomach is of course acid, and this acidity is largely diminished by the advent of the strongly alkaline bile and pancreatic juice, so that the reaction may become alkaline within a short distance of the pylorus. On the other hand the alkaline reaction may not be at all appreciable until the lower end of the intestine is reached, and frequently, at least in dogs, the reaction is faintly acid throughout, whether they are fed on proteids or on a mixture of carbohydrates and fat.[3] The acidity in the latter case is not surprising bearing in mind the readiness with which the carbohydrates undergo a lactic fermentation, especially inside the intestine, and it might therefore have been abnormal in the dog whose food does not normally contain carbohydrates. On the other hand in man, living on a mixed diet, the possibility of a lactic fermentation is always present.[4] It is impossible to make any general statement as to the reaction of the contents of the small intestine ; it varies at different times, and depends upon the kind and relative amount of the several food-stuffs, the changes these undergo and the amount of alkaline secretions with which they are mixed. All the evidence we do possess leads to the belief that intestinal digestion to be of use must be capable of being carried on in a mixture which may be alkaline, or neutral, or even frequently acid. Although the acidity of the intestinal contents may be due to hydrochloric acid in the upper end of the duodenum, the acidity is elsewhere much more probably due to lactic or butyric acids, and it is interesting in this connection to notice that according to Lindberger,[1] the former of these two acids exerts a distinctly favouring influence on tryptic digestion, especially in presence of bile and sodium chloride. Thus in presence of ·02 p. c. lactic acid and 1—2 p. c. bile and sodium chloride fibrin may be digested more rapidly than in a neutral solution and fully as quickly as in a solution of moderate alkalinity. But the presence of ·05 p. c. of lactic acid stops the digestion.

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[1] Kühne, Virchow's *Arch.* Bd. XXXIX. (1867), S. 130. Heidenhain, Pflüger's *Arch.* Bd. X. (1875), S. 570. Mays, *Untersuch. a. d. physiol. Inst. Heidelb.* Bd. III. (1880), S. 378. Lindberger (Swedish). See Abst. in Maly's *Jahresber.* Bd. XIII. (1883), S. 280.

[2] Szabó, *Zt. f. physiol. Chem.* Bd. I. (1877), S. 140. Danilewsky, *Centralb. f. d. med. Wiss.* 1880, No. 51. v. d. Velden, *Deutsch. Arch. f. Klin. Med.* Bd. XXVII. (1880), S. 186. Cf. Langley and Eves, *Jl. of Physiol.* Vol. IV. (1882), p. 19.

[3] Schmidt-Mülheim, *Arch. f. Physiol.* Jahrg. 1879, S. 39. Cash, *Ibid.* 1880, S. 323. Lea, *Jl. Physiol.* Vol. XI. (1890), p. 256.

[4] According to Hammarsten the gastric mucus contains an enzyme which converts lactose (milk-sugar) into lactic acid. See Maly's *Bericht.* Bd. II. (1872), S. 124.

Traces of trypsin have been stated to be found in urine; this is somewhat doubtful.[2]

Trypsinogen.

The zymogen of trypsin. Heidenhain first showed that the pancreas contains, in its absolutely fresh and normal condition, no ready-made enzyme, but an antecedent of the same.[3] This body is readily converted into the active enzyme by the action of dilute acids (1 c.c. of 1 p.c. acetic acid to each 1 grm. of gland-substance) and a conversion also takes place if the gland is kept for some time, especially in the warm, this resulting most probably from the spontaneous acidification which it thus undergoes. The zymogen is soluble in strong glycerine without conversion into the enzyme; it is also soluble in water, in which it is gradually changed into the enzyme, most rapidly when warmed, probably under the influence of the acid reaction which the solution acquires.[4]

Pialyn.[5]

In addition to the two pancreatic enzymes which have already been described, both the secretion and the gland-substance contain a third substance which has not as yet been isolated, of which, therefore, but little is known from a chemical point of view, but which must be regarded as an enzyme in virtue of the typical conditions under which it is able to effect a hydrolytic decomposition of neutral fats into glycerine and free fatty acid. Bernard first drew attention to the existence of this enzyme.[1] It is most actively present in the substance of the *fresh* gland or in its secretion, and may be extracted from the former by means of glycerine or water. In every case it is essential to ensure that the gland had not acquired an acid reaction before extraction, and that all acidification in the extract is absent, since the enzyme is peculiarly sensitive to acids other than fatty, and is readily destroyed by them.[2] Hence a dilute alkaline solution should be employed, and according to Paschutin sodium bicarbonate mixed with the normal carbonate is the most efficient solvent.[3]

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[1] *loc. cit.* ref. 1, on p. 63.

[2] For litt. see ref. 1, sub Pepsin, on p. 61.

[3] Heidenhain, Pflüger's *Arch.* Bd. x. (1875), S. 581. See also Podolinski, *Ibid.* Bd. XIII. (1876), S. 422. Weiss, Virchow's *Arch.* Bd. LXVIII. (1876), S. 413.

[4] Kühne, *Lehrb. d. physiol. Chem.* 1868, S. 120.

[5] From πῖαρ = fat, and λύειν = to split up or decompose.

The presence of the enzyme is tested for by adding the extract to an emulsion of oil of bitter almonds, or other neutral oil or fat, with gum arabic; the mixture is then most carefully neutralised and digested at 40°, together with a minimal amount of neutral sensitive litmus solution. In presence of the enzyme the mass turns more or less rapidly red, owing to the liberation of the free fatty acid.

The enzymic nature of the active agent is shown by the fact that its activity is greatest at about 40°, is destroyed by boiling, and is dependent upon the reaction of the digestive mixture, being greatest in presence of a dilute alkali, although it will show itself in a neutral solution. It will also be observed that the decomposition which pialyn effects is typically hydrolytic.

Rennin.

Extracts of the mucous membrane of the stomach of young animals, and more especially of the calf, have been known from time immemorial to possess a most remarkable power of causing milk to clot, and rennet was commonly employed by the Romans for the manufacture of cheese. The active agent in producing the clot was in more recent times supposed to be either the acidity of the extract itself or the production of lactic acid from milk-sugar (lactose) by means of some active principle in the extract. Heintz and Hammarsten, however, showed that this view is untenable; and we now know that the substance to which the clotting is due is an enzyme to which the name of *rennin* may be conveniently given.[4] The enzymic nature of the active agent in rennet is clearly shown by the typical relationship which it exhibits in its activity to the reaction of the solution in which it is present,[5] to the temperature at which its activity is greatest, to the fact that the briefest exposure to 100° or the more prolonged exposure to lower temperatures (70° or above)[1] suffices to destroy its active properties, and to the fact that a minute trace suffices to clot a relatively enormous amount of casein.[2]

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[1] *Compt. Rend.* T. xxviii. (1849), p. 249. See also his *Leçons de physiol. expér.* T. ii. (1856), p. 253.

[2] Grützner, Pflüger's *Arch.* Bd. xii. (1876), S. 302.

[3] *Arch. f. Anat. u. Physiol.* Jahrg. 1873, S. 386.

[4] This name seems more convenient than the more commonly used expressions 'the rennet ferment' or 'the milk-curdling ferment.'

[5] Hammarsten (Swedish). See Abst. in Maly's *Bericht.* Bd. ii. (1872), S. 121. Heintz, *Jn. f. prakt. Chem.* (N.F.) Bd. vi. (1872), S. 374. See also Al. Schmidt.

Nothing is known as to the chemical nature of rennin. Extracts of the gastric mucous membrane contain both rennin and pepsin. Hammarsten[3] has obtained it free from the latter enzyme by fractional precipitation with either magnesium carbonate or normal lead acetate, by which pepsin is more readily precipitated than is rennin. He further endeavoured to separate out the enzyme, after freeing it from pepsin, by precipitation with the acetates of lead in presence of a trace of ammonia; this precipitate was then carefully decomposed with very dilute sulphuric acid, and the enzyme finally separated by means of cholesterin. (Vide preparation of pepsin, p. 59.) The reactions of the purified enzyme described by Heidenhain seem to indicate that it is not a proteid.

Aqueous and glycerin extracts of the gastric mucous membrane are usually found to be active in clotting milk,[4] but the activity of a faintly acid extract is in all cases greater. This is due to the existence of a rennin zymogen (renninogen) which is readily converted into the enzyme by the action of acids.[5] The preparation of highly active and permanent solutions of rennin is of considerable commercial importance in connection with the cheese-making industry. The most efficient extractive is sodium chloride, 5—15 p.c.; and permanency is attained by the addition of alcohol, or in some cases thymol.[6]

Although rennin is most copiously present in the gastric mucous membrane of the calf, it may be obtained from the tissue of almost any stomach, if not as ready-made enzyme, at least in the form of a zymogen (Hammarsten). It occurs also in the stomach of children[7] and of man;[8] and Roberts has described a similar enzyme in the pancreas of the pig, ox, and sheep.[9] Rennin is stated to occur in traces in urine.[10]

Maly's *Bericht.* Bd. IV. (1874), S. 159. Langley, *Jl. of Physiol.* Vol. III. (1881), p. 259.

[1] Mayer, *Die Lehre von dem chem. Fermenten*, 1882. See also Maly's *Ber.* Bd. X. (1880), S. 208.

[2] 400,000—800,000 times its own weight. Hammarsten. See Maly's *Bericht.* Bd. VII. (1877), S. 166.

[3] Maly's *Bericht.* Bd. II. S. 121. See also Friedberg, *Jl. Amer. Ch. Soc.* May, 1888, p. 15.

[4] Hammarsten, *loc. cit.* See also Erlenmeyer, *Sitzb. d. k. b. Akad. d. Wiss.* München, 1875, Hft. 1.

[5] Hammarsten, *loc. cit.* Langley, *Jl. of Physiol.* Vol. III. (1881), p. 287.

[6] Soxhlet, *Milchzeitung*, 1877, Nos. 37, 38. *Chem. Centralb.* 1877, S. 745. Nessler, *Landwirth. Wochenblatt. f. Baden*, 1882, S. 57. Friedberg, *Jl. Amer. Ch. Soc.* May, 1888, p. 15. Ringer, *Jl. of Physiol.* Vol. XII. (1891). Note 2, p. 164.

[7] Zweifel, *Centralb. f. d. med. Wiss.* 1874, No. 59. Hammarsten, Ludwig's *Festgabe*, Leipzig, 1875.

[8] Schumberg, Virchow's *Arch.* Bd. XCVII. (1884), S. 260. Boas, *Centralb. f. d med. Wiss.* 1887, No. 23.

[9] *Proc. Roy. Soc.* No. 29, 1879, p. 157.

[10] See Helwes, Pflüger's *Arch.* Bd. XLIII. (1888), S. 384.

Fibrin-ferment.

Buchanan's work (1831) on the clotting of blood, more particularly his experiments with 'washed clot,' when examined in the light of our present knowledge, shows clearly that he was in reality dealing with that factor in the whole process which was independently discovered by Alexander Schmidt and more specifically described by him in 1872 under the name of 'fibrin-ferment.'[1] Its existence had been foreshadowed in some experiments made by Brücke, in which he showed that the fibrinoplastic action of precipitated paraglobulin was partly, at least, dependent upon the admixture of some other substance, which he regarded as the truly fibrinoplastic factor. Thus, he showed among other things that the more a serum is diluted before the paraglobulin is precipitated from it by means of CO_2, the less marked are its fibrinoplastic powers.[2] Further, Mantegazza had in 1871 put forward the view, also held by Buchanan, that the white corpuscles play some important part in the formation of fibrin, without in any way characterising the substance which he suggested was probably discharged from them as the determinant of the whole process.[3] The time was thus ripe for Schmidt's discovery.[4] He prepared the ferment by precipitating serum with 15—20 volumes of strong alcohol; the precipitate was treated for *at least* 14 days with the alcohol to insure complete (?) coagulation and insolubility of the proteids; after which time it was removed by filtration, dried in vacuo over sulphuric acid, pulverised, and extracted with distilled water in volume equal to twice that of the serum originally employed. The ferment solution thus obtained is by no means pure, and not very active. More recently Hammarsten has obtained the ferment in solution free from paraglobulin.[5] He saturates serum with magnesium sulphate at 30°, and filters off the precipitated paraglobulin at the same temperature. The filtrate he dilutes with nine volumes of water, and to this adds gradually, and with continuous stirring, dilute caustic soda until a permanent, flocculent, and fairly copious precipitate is formed. This precipitate carries the ferment down mechanically, and is finally washed, pressed, suspended in water, dissolved by acetic acid to a neutral solution, and dialysed till free from salt.

For ordinary purposes an extremely active ferment solution may be most readily obtained by Gamgee's method of extracting the so-called 'washed blood clot' with 8 p.c. solution of sodium chloride.[6] The solution in this case contains a large amount of globulins in solution, as also does the similar extract which may be equally efficiently prepared from ordinary washed fibrin.[1]

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[1] An account of Buchanan's experiments has been given by Gamgee. *Physiol. Chemistry*, Vol. I. 1880, p. 43. See also *Jl. of Physiol.* Vol. II. (1879), p. 145.

[2] *Sitzb. d. Wien. Akad.* Bd. LV. (2 Abth.), 1867, S. 891.

[3] See Abst. in Maly's *Bericht.* Bd. I. (1871), S. 110.

[4] Pflüger's *Arch.* Bd. VI. (1872), S. 457.

[5] *Ibid.* Bd. XVIII. (1878), S. 89; XXX. (1883), S. 457.

[6] Gamgee, *Jl. of Physiol.* Vol. II. (1879), p. 150.

In no case as yet has the fibrin-ferment been obtained in a condition of such purity as to justify any dogmatic statement as to its chemical composition. All the solutions whose preparation has been described above yield strong proteid reactions, and Halliburton[2] has argued from his own experiments and a criticism of preceding work that the ferment is really a proteid identical (?) with what he had previously called 'cell-globulin' (*antea*, p. 28). On the other hand it is possible by appropriate methods to free the salt-extracts of fibrin very completely from proteids without any great loss of ferment activity, certainly without any such loss as would necessarily be the case if the active substance were a globulin.[3] It may be said that the apparent ferment-powers in such cases are in reality due to the presence of calcium sulphate, which is now known to promote the clotting of a dilute salt-plasma to an extraordinary degree;[4] but as against this the fact may be quoted that solutions free from proteid reaction, and which had been freed from salts by careful dialysis, lost their activity on heating to 60—70°, which they would not have done had the activity been due merely to calcium sulphate.

When Schmidt's method is applied to blood received directly from an artery into an excess of alcohol no ferment can be obtained from the precipitate thus obtained. It is hence evident that the living, circulating blood contains no preformed ferment, and the question thus arises from what does it take its origin during the clotting of blood and presumably as an immediate antecedent to that clotting? Buchanan held distinctly the view that the active agent in the whole process was in some way connected with, if not derived from, the white corpuscles, a view also held later on by Mantegazza. Schmidt also took this view, basing it on an elaborate series of investigations for which his original works must be consulted.[5] Löwit, experimenting with lymph as well as blood, while denying that the white corpuscles break down at clotting in the way Schmidt described, still connects them with the production of the initiative factor in the whole process.[6] Still further evidence in the same direction may be derived from the experiments of Rauschenbach[7] and Halliburton,[8] and of Fano, who observed that when peptone-plasma is freed as completely as possible from white corpuscles it cannot be made to clot in the usual way by the addition of water.[1]

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[1] Lea and Green, *Jl. of Physiol.* Vol. IV. (1883), p. 386.

[2] *Jl. of Physiol.* Vol. IX. (1888), p. 265.

[3] Lea and Green, *loc. cit.*

[4] Green, *Ibid.* Vol. VIII. (1887), p. 354.

[5] Pflüger's *Arch.* Bd. IX. (1874), S. 353; XI. (1875), Sn. 291, 515.

[6] *Sitzb. d. Wien. Akad.* (2 Abth.), Bd. LXXXIX. (1884), S. 270; XC. S. 80.

[7] *Inaug.-Diss.* Dorpat, 1883. See also the Dissertations (Dorpat) of F. Hoffmann, 1881. Samson-Himmelstjerna, 1882; Heyl, 1882.

[8] *loc. cit.* See also Krüger, *Zt. f. Biol.* XXIV. (1888), S. 189.

In addition to the red and white corpuscles blood also contains, as already described (§ 33), a third structural element, the 'platelets,' and several observers have endeavoured to connect the first cause of the clotting of blood with some breaking down and disappearance of these structures.[2] This view is as yet insufficiently supported, and is combated by several observers; [3] bearing in mind however how little is known about the origin and nature of these platelets the question of their relationship to blood-clotting must still be regarded as awaiting a decisive answer.

In addition to the undoubted relationship of leucocytes to fibrin-formation it appears that the protoplasm of many other cells, both animal and vegetable, may exert an influence similar to that of the white corpuscles of blood.[4]

Wooldridge regarded the leucocytes as entirely secondary and very subordinate factors in the process of clotting, as also the fibrin-ferment. According to his view blood-plasma contains in itself all the elements requisite for the formation of fibrin, which he considers to be in no sense the outcome of any fermentative process. He described three coagulable proteids *A*- *B*- and *C*-fibrinogen. The last of these occurs in minimal quantities in plasma, is identical with the substance ordinarily known as fibrinogen, and clots on the addition of fibrin-ferment. According to his view clotting is due to a transference of lecithin from its combination with *A*-fibrinogen to *B*-fibrinogen, by which means both the fibrinogens disappear and fibrin takes their place.[5]

The information which we possess as to the nature of the fibrin-ferment is much less complete and satisfactory than in the case of other enzymes. But that it is properly placed in the class of these substances is shown by the typical facts that its activity is closely dependent upon temperature, being destroyed by heating to 70° ; that it does not affect the amount but only the rate of change of fibrinogen into fibrin ; that it is carried down by gelatinous precipitates formed in its solutions (Hammersten), produces a change which is out of all proportion to the mass of enzyme employed, and is not, so far as we know, used up in the change which it induces, since it is present in serum.

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[1] *Arch. f. Physiol.* Jahrg. 1881, S. 288. *Centralb. f. d. med. Wiss.* 1882, S. 210.

[2] Hayem, *Gaz. med. de Paris,* 1878, p. 107. *Compt. Rend.* T. LXXXVI. (1878), p. 58. *Arch. de Physiol.* 1878, p. 692. Bizzozero, Virchow's *Arch.* Bd. XC. (1882), S. 261. Laker, *Sitzb. d. Wien. Akad.* (2 Abth.), Bd. LXXXVI. (1883), S. 173. Hayem's colourless 'hæmatoblasts' are identical with Bizzozero's 'platelets.' The true hæmatoblasts are the cells described by Neumann, Rindfleisch, and others as occurring in the red marrow of bones.

[3] Fano, *Centralb. f. d. med. Wiss.* 1882, S. 210. Löwit, *loc. cit.* Schimmelbusch, Virchow's *Arch.* Bd. CI. (1885), S. 201.

[4] Rauschenbach, *loc. cit.* Gröhmann, *Inaug.-Diss.* Dorpat, 1884.

[5] Croonian Lecture, *Roy. Soc. Lond.* 1886. Ludwig's *Festschrift,* 1887. See also Halliburton, *loc. cit. antea.*

Muscle-enzyme.

The phenomena of the clotting of muscle-plasma compared with those of blood-plasma and the relationship of the process to the presence of neutral salts and to temperature suggest at once that the change is probably one in which some enzyme plays a part. Immediately after Schmidt's discovery of the fibrin-ferment the question of the existence of a myosin-ferment was investigated under his guidance,[1] and resulted in the discovery of the existence in muscles of an enzyme which appeared to be identical with fibrin-ferment rather than specifically myosinic. The later work of the Dorpat School further confirmed the above, but failed to establish the existence of an enzyme, differing from fibrin-ferment and specifically active in promoting the clotting of muscle-plasma.[2] More recently it has been shown that by applying Schmidt's method to muscles which have been treated for some time with alcohol, a solution may be obtained which hastens the clotting of diluted muscle-plasma, does not facilitate the formation of fibrin in blood-plasma, and, unlike fibrin-ferment, requires to be heated to 100° before it loses its activity.[3] The active agent in the solution is therefore not identical with fibrin-ferment and may be spoken of as a myosin-ferment.

Urea-ferment.

When urine is exposed to the air its acidity at first increases, but in most cases this speedily gives way to a marked alkalinity, which is accompanied by the evolution of ammonia. This is due to a hydrolytic fermentative change resulting from the appearance and development in the urine of certain micro-organisms of which the best known is the Torula ureae.[4] Normally urine is free from these organisms and may be kept in the excised bladder for an indefinite period without exhibiting any tendency to become alkaline;[5] in certain abnormal conditions it may undergo an active alkaline fermentation while still in the bladder. The part played by the organisms was for a long time regarded as similar to that of yeast-cells in promoting alcoholic fermentation. Soon however evidence was adduced which showed that the change was not necessarily due solely to the life and growth of the organisms in the solution, for it was found that the fermentation might be very complete in presence of an amount of carbolic acid which is fatal to the development of micro-organisms.[1] The probable existence of an enzyme as a possible factor in the whole process which was thus demonstrated was reduced to a certainty by the experiments of Musculus.[2] Employing the thick mucous excretion of urinary catarrh he precipitated the mucin with alcohol, dried the precipitate at a low temperature, extracted it with water and found the extract to possess active hydrolytic powers in a solution of urea. The proof of the existence of the enzyme in a pathological mucous urine in which there is frequently no reason to suspect the existence of any micro-organisms still left open the question of the isolation of the enzyme from the micro-organism itself. When urine which by exposure to the air has entered into active alkaline fermentation and, as shown by microscopic examination, is full of Torulae, is efficiently filtered no enzyme capable of hydrolising urea can be precipitated by alcohol from the clear filtrate. If on the other hand the unfiltered urine be precipitated with an excess of alcohol and the precipitate washed with alcohol and dried in the air, a powder is obtained which is itself extraordinarily active, and yields to an aqueous extract a soluble enzyme which rapidly converts urea into ammonia and carbonic acid. The rapidity of the conversion precludes the intervention of any developing organism, and that the change is truly due to an enzyme is shown by the fact that it takes place with equal readiness in presence of chloroform.[3]

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[1] Michelson, *Diss.* Dorpat, 1872.

[2] Grubert, *Diss.* Dorpat, 1883. Klemptner, *Ibid.* Kugler, *Ibid.*

[3] Halliburton, *Jl. of Physiol.* Vol. VIII. (1887), p. 159.

[4] Müller, *Jn. f. prakt. Chem.* Bd. LXXXI. (1860), S. 467. Pasteur, *Compt. Rend.* T. L. 1860, p. 869. van Tieghem, *Ibid.* T. LVIII. 1864, p. 210. But see also Jaksch, *Zt. f. physiol. Chem.* Bd. V. (1881), S. 395. Leube, Virchow's *Arch.* Bd. C. (1885), S. 540. Miquel, *Bull. de la Soc. Chim.* T. XXIX. (1878), p. 387; XXXI. p. 391; XXXII. (1879), p. 126.

[5] Cazeneuve et Livon, *Compt. Rend.* T. LXXXV. (1877), p. 571. *Bull. de la Soc. Chim.* T. XXVIII. (1877), p. 484.

It is of some interest to notice here that from what has been said above the organisms to whose activity the fermentation is due do not discharge their enzyme into the surrounding medium; when killed however, as by means of alcohol, they yield it readily to a suitable extractive. This holds good also in the case of invertin, which is not found in the filtrate from yeast, while it may readily be extracted from the cells when killed by ether or alcohol.[4] Similarly it appears that putrefactive bacteria may excrete or yield an enzyme whose action is closely analogous to that of trypsin.[5]

The most prolific source of the urea enzyme is in all cases the mucous urine passed in inflammatory conditions of the bladder.

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[1] Hoppe-Seyler, *Med.-chem. Untersuch.* Hft. 4, 1871, S. 570.

[2] *Compt. Rend.* T. LXXVIII. (1874), p. 132; LXXXII. (1876), p. 334. Pflüger's *Arch.* Bd. XII. (1876), S. 214. See also Lailler, *Compt. Rend.* T. LXXVIII. p. 361.

[3] Lea, *Jl. of Physiol.* Vol. VI. (1885), p. 136.

[4] Hoppe-Seyler, *Ber. d. deutsch. chem. Gesell.* 1871, S. 810. Confirmed by Lea For chemistry of invertin see Donath, *Ber. d. deutsch. chem. Gesell.* 1875, S. 795; 1878, S. 1089. Barth, *Ibid.* 1878, S. 474. Kjeldahl (Danish). See Abst. in Maly's *Bericht.* 1881, S. 448. Mayer, *Zt. f. Spirit-Indust.* 1881, Nos. 16, 22. Löw, Pfluger's *Arch.* Bd. XXVII. (1882), S. 203.

[5] Hüfner, *Jn. f. prakt. Chem.* (N.F.) Bd. V. (1872), S. 372. Herrmann, *Zt. f. physiol. Chem.* Bd. XI. (1887), S. 523. E. Salkowski, *Zt. f. Biol.* Bd. XXV. (1889), S. 92.

In this case the enzyme appears to be closely associated with the mucin and is presumably a secretory product of the mucous membrane, for it is frequently obtained when there has been no operative use of surgical instruments which could account for the introduction of micro-organisms from the exterior.

In concluding this account of the more important enzymes of the animal body it may not be out of place to say a few words on the probable mode of action of the ferments and enzymes.

The term fermentation was applied originally to the changes, accompanied by characteristic frothing, foaming, and evolution of gases, which saccharine solutions such as the expressed juice of fruits or infusions of grain undergo on exposure to the air. The chemical changes and products of the fermentation were studied from the earliest times, and in 1680 Leuwenhœk described, with the aid of the newly-invented microscope, the small, spherical particles which are now known as yeast-cells, to be the exciting cause of the whole process. He did not however ascribe any organisation to these particles, and it was not until 1835 and 1837 that Cagniard de Latour and Schwann respectively but independently took up the investigation where Leuwenhœk had left it, and established firmly and finally the organised and plant-like nature of the yeast-cell and the absolute dependence of fermentation upon its presence in the fermenting fluid.[1] The yeast-cell having thus been definitely recognized as the cause of the fermentation, the interesting question at once arose as to how the known cause produces the observed effect, and to this question many answers have been given, of which the following are the more important.

Liebig regarded the ferments as substances in a state of progressing decomposition during which the equilibrium of their constituents is upset and a rapid motion of their minuter parts established. When brought into contact with other decomposable substances the motion of the ferment's particles is communicated to the former, whereupon it also undergoes a decomposition resulting in the formation of the simpler products which make their appearance and are characteristic of the fermentation. According to this view the organised nature of the yeast-cells is left out of account and the phenomena attributed entirely to the purely chemical decomposition of their constituent substance, set going at the outset by oxygen.[2] Pasteur regarded alcoholic fermentation as indissolubly connected with the vegetative growth, multiplication, and metabolism of the yeast-cell. According to this view sugar is the food-stuff out of which the organism obtains the material requisite for its metabolism and growth, the products of the fermentation being thus, as it were, the excretionary residues of the metabolised food.[1] A third view attributes the fermentative decomposition to the production by the organised ferments of soluble unorganised enzymes to whose activity the decomposition is due. This view received its chief support from the discovery that a part at least of the change which sugar undergoes in presence of yeast may be obtained by means of the soluble enzyme 'invertin' which can readily be extracted from the dead cells.[2] But as yet all efforts to obtain an enzyme capable of carrying the decomposition beyond the initial stage of inversion have been fruitless. According to von Nägeli the living substance of the organised cell is to be regarded as being in continuous and rapid molecular vibration, and the decomposition of the fermentable substance as the result of the direct transference of these vibrations to this substance, by means of which its equilibrium is upset and it is split up into simpler and therefore more stable products.[3] To discuss the merits of these various theories and the experiments upon which they are based is quite impossible within any reasonable limits of brevity. We shall perhaps be not far wrong in considering that as regards the organised ferments the changes they effect may be, in their earlier stages, partly the outcome of the action of some soluble enzyme, and partly the result of that cycle of metabolic (chemical) processes which occur continuously in their protoplasm, in virtue of which they are spoken of as 'living.' Similarly in the higher animals we find a large number of simpler processes carried on by means of isolable enzymes, by which undoubtedly the labours of the protoplasm in performing its own more complicated activities are materially lightened. But we are still face to face with numberless decompositions which cannot as yet be reproduced outside the limits of living matter and which cannot be explained with reference to anything other than the direct activity of living matter.

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[1] Erxleben in 1818 had described and spoken of yeast as a vegetative organism, as also in 1825 had Desmazières, who ascribed to it an animal rather than vegetable nature.

[2] *Ann. d. Chem. u. Pharm.* Bd. xxx. (1839), Sn. 250, 363. Stahl in 1734 had expressed practically identical views.

The general conditions and factors which characterise the action of the soluble ferments or enzymes have already been mentioned (p. 53), but without making any suggestion as to the probable way in which they produce and carry on the decompositions to which they give rise. Liebig's theory of the mode of action of yeast, since it left the organisation and life of the cell entirely out of account, and was based simply upon the supposed properties of the changing cell-substance, might obviously therefore be applied to any ordinary soluble enzyme. There is however no evidence to show that the enzymes are in the state of change or decomposition which Liebig supposed; on the contrary they are observed to be on the whole remarkably stable substances, from the point of view that a minute trace can produce a profound decomposition in a relatively enormous mass of material, during an almost indefinitely long time, without itself undergoing any proportionate alteration or destruction.[1] The theory of v. Nägeli previously quoted was applied by its author to explain the fermentative power of the *living* cell, and is thus not directly applicable to the non-living enzymes. Mayer, it is true, has put forward a view which is essentially a development of v. Nägeli's and is applicable to the enzymes. These substances are in all cases produced solely and entirely by the activity of living cells or organisms, and Mayer regards them as retaining in themselves a portion of that molecular motion which is supposedly so characteristic of the living parent cell from which they have been separated.[2] It cannot however be said that these theories afford any real insight into the probable mode of action of an enzyme, and we must look for it in some other direction.

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[1] This view was keenly attacked by Liebig, *Ann. d. Chem. u. Pharm.* Bd. CLIII. (1870), Sn. 1, 137. See Pasteur in reply, *Ann. Chim. Phys.* 4 Sér. T. XXV. (1872), p. 145.

[2] The inverting power of yeast was first stated by Dubrunfaut in 1847. Berthelot obtained invertin in solution in 1860, and Hoppe-Seyler prepared it in the form of a soluble powder in 1871. See references on p. 71.

[3] *Theorie der Gährung*, München, 1879.

Attention has been already drawn (p. 53) to the existence of a large and increasing class of chemical reactions whose occurrence is determined by mere traces of some substance which does not itself at the same time undergo any change during the decompositions which it initiates, and the enzymes have been compared to these substances. Now in the case of the reactions of which we are now speaking it is known in some and probable in all that the process which takes place is in general terms the following. The determinant substance interacts with one of the reagents to form a compound which can now enter into combination with the other; the result is the formation of a more complex compound which at once decomposes, giving rise to products of which one is the original determinant substance in an unaltered form, the others the product characteristic of the reaction.[3] This suggests at once that the enzymes may play their part in a manner similar to that of the determinant in the above reactions, a view which has been put forward but scarcely receives the attention that it deserves.[4]

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[1] Berzelius explained fermentation as the outcome of a mysterious 'catalytic action,' or 'action by presence' or 'contact.' He thus compared ferments to platinum-black, which is able, in minute quantity, to cause a liberation of oxygen from peroxide of hydrogen without itself undergoing any recognisable change. This is however no explanation, for it does not amount to more than saying that given the contact of two substances capable of reacting on each other, a certain reaction takes place.

[2] *Die Lehre von den chem. Fermenten*, Heidelb. 1882.

[3] Vide the reactions in the continuous etherification process and the manufacture of sulphuric acid. See also Traube (*Ber. d. deutsch. chem. Gesell.* 1885, S. 1890), on the part played by water in determining the explosion of O and CO.

[4] Kühne, *Lehrb. d. physiol. Chem.* 1868, S. 39. Hoppe-Seyler, *Med.-chem. Unters.*

In most cases it is known, and it is probable in all, that the soluble ferments act by bringing about a union of water with the substances upon which they act. This process might be supposed to take place in the following way. The enzyme uniting with the substance to be decomposed, the compound thus formed is now able to unite with water, and this final more complex and hence less stable compound undergoes a decomposition of which the original enzyme is one product, the others being the hydrated and hence altered substance whose formation is characteristic of the whole process. It is impossible within convenient limits to bring forward here all the direct evidence in favour of the above view as to the mode of action of enzymes; it must suffice to say that as regards pepsin there is some reason for thinking that it can enter into combination with hydrochloric acid. Finally it may be stated that the characteristic phenomena of zymolysis in connection with the influence of heat, the effect of various salts and dilution, the cessation of the change in presence of an excess of the products of that change, &c., are such as careful consideration shows might from several points of view be expected on the supposition that the above theory of enzyme action is true.

Chemical action is in all cases accompanied by an evolution or absorption of heat, and it will add to the completeness of this account of the ferments if we consider briefly the heat-phenomena which accompany the chemical action due to the enzymes. Liebig regarded the fermentative decomposition of sugar as necessitating a considerable consumption of energy; which he supposed to be derived from the decomposing albumin of the ferment-substance. Hoppe-Seyler on the other hand put forward the general view that heat is evolved in every case of ferment action, basing it upon experiments in which he observed a distinct rise of temperature during the action of pancreatic extracts upon starch, but more particularly upon the opinion that the heat of combustion of the products of zymolysis is in all cases less than that of the original substance from which the products have been formed.[1] And this is undoubtedly the correct view. In addition to Hoppe-Seyler other observers have observed a rise of temperature during zymolysis, *e. g.* in the case of the formation of fibrin,[2] the clotting of milk,[3] and the inversion of cane-sugar.[4] Maly on the other hand observed a considerable absorption of heat during the action of pepsin on proteids and ptyalin on starch.[5] These experiments it may be observed are discordant, and in reality they neither speak strongly for nor against the evolution of heat during the action of the enzymes; as

Hft. 4, 1871, S. 573. v. Wittich, Pflüger's *Arch.* Bd. v. (1872), S. 435. Wurtz, *Compt. Rend.* T. xci. (1880), p. 787.

[1] *Med.-Chem. Unters.* Hft. 4, 1871, S. 574.

[2] Lépine, *Gaz. Méd.* Paris, 1876. No. 12.

[3] Mayer, *Milchzeitung*, 1881, No. 2, 3, 4, 6. See Abst. in Maly's *Bericht.* 1880, S. 209. But see also Musso, *Ibid.* 1879, S. 16.

[4] Kunkel, Pflüger's *Arch.* Bd. xx. 1879, S. 509. But see Nägeli, *Ibid.* Bd. xxii. S. 310.

[5] Pflüger's *Arch.* Bd. xxii. (1880), S. 111.

a matter of fact they could scarcely be expected to do so, since it is extremely difficult to make allowance for the heat which may be simply absorbed or set free as the result of the varying solubilities of the original substance and the products of its decomposition. The real proof of the correctness of Hoppe-Seyler's view is the fact, already stated, that the heat of combustion of the products of zymolysis is less than that of the substance from which they are derived.[1]

NITROGENOUS NON-CRYSTALLINE BODIES ALLIED TO PROTEIDS.

These resemble the proteids in many general points, but exhibit among themselves much greater differences than do the proteids. As regards their molecular structure nothing satisfactory is known. Their percentage composition approaches that of the proteids, and like these they yield, under hydrolytic treatment, large quantities of leucin and in some cases tyrosin. They are all amorphous.

Mucin.

This is the substance which gives to many animal secretions, such as saliva, bile, synovial fluid, &c., their characteristic ropy consistency. It may also be obtained by the use of appropriate solvents from the tissues themselves, such as submaxillary gland, tendons, and umbilical cord. It is peculiarly copious in the secretion which may be collected on stimulating the mantle of Helix pomatia, or in an extract of the tissues of this animal. The general phenomena of the formation of mucin by mucous cells, and more particularly the characteristic behaviour of the mucous granules in relation to the secretory activity of the sub-maxillary gland,[2] leave but little doubt that mucin is to be regarded as derived from the true proteids; in conformity with this it yields many of the reactions characteristic of the proteids (Millon's and xanthoproteic), and by the action with mineral acids some form of acid-albumin is usually obtained. During this treatment (or with alkalis) moreover a second product generally makes its appearance, which belongs to the group of carbohydrates and by heating with acids may be made to yield a reducing sugar. Notwithstanding the views which have frequently been advanced that mucin is in reality a mixture of proteid and carbohydrate material, it is now known with considerable certainty that it is a unitary substance which, from what has been already said, might be almost regarded as an animal glucoside. It further appears that the substance at first secreted by the mucous cells (of Helix) may not be typical mucin, but a sort of mucinogen which readily gives rise to mucin on treatment with dilute (·01 p.c.) caustic potash.[1] If it be assumed for the moment that there is only one kind of mucin, then the following general statements as to this substance may be additionally made. It is precipitated from its solutions by acetic or hydrochloric acids, the precipitate being soluble in excess of the latter but not of the former acid. In its precipitated form it swells up strongly in water but does not go into true solution; the addition of dilute alkalis (·1—·2 p.c.) or of lime-water leads to its ready solution, from which it can again be precipitated by the addition of an acid. It may be extracted from any mucigenous tissue by the use of dilute alkalis or lime-water, and in solution is somewhat characteristically precipitated by basic lead acetate. Our knowledge of mucin is however in an extremely transitional condition, and recent investigations have shown that probably the mucins derived from different sources are really distinct substances, just as we are familiar with different forms of proteids. From this it follows that no general statement of the properties of the mucins can be as yet made which would be other than misleading, and it will conduce to clearness to give a brief account of this substance as obtained from each of the chief sources from which it has been derived.

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[1] For heat of combustion of physiologically important substances see Rechenberg. *Inaug. Diss.* Leipzig, 1880, and *Jn. f. prakt. Chem.* (N. F.) Bd. XXII. (1880), Sn. 1, 223. See also Stohmann, *Ibid.* Bd. XXXI. (1885), and Landwirth Jahrb. Bd. XIII. S. 513. Rubner, *Zt. f. Biol.* Bde. XIX. (1883), S. 313; XXI. Sn. 250, 337. Berthelot et André, *Compt. Rend.*, T. CX. (1890), p. 884.

[2] Langley, *Jl. of Physiol.* Vol. X. (1889), p. 433.

The mucin of bile.[2] Mucin is not a constituent of normal bile when freshly secreted, but is found in it as the result of the secretory activity of the internal epithelium of the gall-bladder. It is best prepared as follows (Paijkull). Bile is mixed with five volumes of absolute alcohol and centrifugalised; the precipitated mucin which is thus obtained is then dissolved in water and the above process repeated two or three times. An aqueous solution of this mucin is precipitated by acetic and hydrochloric acids, is soluble in excess of either acid, and yields strongly marked proteid reactions. This mucin differs from that obtained from other sources in not yielding any reducing substance when boiled with acids, and in the solubility of its precipitate obtained by means of acetic acid in an excess of this acid. It also contains phosphorus, and is by some regarded as more closely allied to the nucleo-albumins (see p. 89) than to the true mucins.

The mucin of the sub-maxillary gland.[3] The gland is finely minced, washed, and extracted with water: the extract is filtered and hydrochloric acid is added up to ·1—·15 p.c. The mucin is thus precipitated at first, but at once passes into solution, from which it is precipitated by the addition of a volume of water equal to three to five times that of the original solution. This precipitate is then again dissolved in dilute hydrochloric acid and reprecipitated by water, the process being repeated several times. As thus prepared and thoroughly washed it possesses a distinctly acid reaction; it may be dissolved to a neutral solution by the *cautious* addition of *very dilute* alkalis, and now exhibits the following properties. It is readily precipitated by acetic acid, much less readily in presence of sodium chloride; this salt on the other hand greatly facilitates the precipitation of mucin by alcohol, which again does not take place in presence of a trace of free alkali. Any excess of alkali, especially on warming, at once changes the substance so that its characteristic ropiness is permanently lost, and boiling with dilute mineral acids yields a reducing substance. It gives the usual reactions for proteids and is strongly precipitated by the acetates of lead and by $CuSO_4$ and by excess of NaCl and $MgSO_4$.

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[1] Hammarsten, Pflüger's *Arch.* Bd. XXXVI. (1885) S. 390.

[2] Landwehr, *Zt. f. physiol. Chem.* Bd. V. (1881), S. 371; VIII. (1883), S. 114. Paijkull, *Ibid.* XII. (1887), S. 196.

[3] Hammarsten, *Zt. f. physiol. Chem.* XII. (1888), S. 163. Contains references to other literature. Obolensky, Hoppe-Seyler's *med.-chem. Unters.* Hfl. 4 (1871), S. 590. Also in Pflüger's *Arch.* Bd. IV. (1871), S. 336.

The mucin of Helix pomatia.[1] Hammarsten distinguishes between the mucin contained in the secretion of the mantle and that which may be derived from the foot of this animal. *Mantle-mucin.* The secretion of the mantle contains a mucigenous substance precipitable by acetic acid which is exceedingly insoluble in water, but is readily converted into true mucin by the action of dilute (·01 p.c.) caustic potash. From its solution in alkali it may be purified by precipitation with acetic acid, washing, resolution in alkali and reprecipitation with acid. When dissolved in a trace of alkali the solution yields the reactions typical of other mucins, but it differs from these in the fact that the precipitate formed on the addition of hydrochloric acid (or acetic) is not soluble in excess of the acid. *Foot-mucin.* It may be obtained by extracting the foot with .01 p.c. KHo; from this solution it is now precipitated by the addition of hydrochloric acid (not acetic) up to ·1 – ·2 p.c., redissolved in alkali and reprecipitated with acid, the process being repeated several times. Solutions of this mucin resemble those of mantle-mucin in all essential respects, the only difference which is stated to be characteristic of the two being that in presence of sodium chloride, mantle-mucin, like that of the submaxillary gland, is not precipitated by faint acidulation with acetic acid, whereas under similar conditions solutions of foot-mucin cannot even be neutralised without yielding an opalescence or precipitate.

The mucin of tendons.[2] The tendo Achillis of the ox is cut into thin slices, washed with distilled water and extracted with half-saturated lime-water; the mucin is thus dissolved, and is purified by precipitation with either acetic or hydrochloric acids, re-solution in dilute alkali, and reprecipitation with acids. In its general reactions it resembles the mucins previously described, but appears to differ from them in its distinctly greater resistance to the action of acids and alkalis.

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[1] Hammarsten, Pflüger's *Arch.* Bd. xxxvi. (1885), S. 373. Gives previous literature.

[2] Löbisch, *Zt. f. physiol. Chem.* Bd. x. (1886), S. 40. Gives previous literature.

Mucin of the umbilical cord.[1] May be extracted by means of water and is readily precipitated from the solution by acetic acid. It appears to differ from the other mucins in containing more nitrogen and a considerable amount of sulphur: it lies in fact somewhat midway between the proteids and true mucins.

By prolonged boiling with sulphuric acid mucins yield leucin and tyrosin, but the products of their decomposition have not been as yet fully studied.[2]

Analyses of the several mucins exhibit differences in percentage composition which lie within somewhat similar limits to those already assigned (p. 5) to the proteids. A comparison of these seems to justify the statement that on the whole the mucins contain slightly less carbon and distinctly less nitrogen than do the proteids.[3]

During his researches on mucin Landwehr[4] obtained a substance to which he gave the name of "animal-gum" from its general similarity to the vegetable products of the same name. He was at first inclined to regard the mucins as mixtures of this carbohydrate with other proteid substances, but this view he subsequently modified.[5] Further investigation has led him to regard animal-gum as occurring in many tissues of the body, and to speculate on its physiological and pathological significance.[6] Its isolation from the several tissues is somewhat lengthy and complicated, and for this Landwehr's original papers must be consulted. It dissolves in water to form a readily foaming solution, from which it may be precipitated by alcohol. In alkaline solution it readily dissolves cupric oxide which is not reduced on boiling: when boiled with dilute mineral acids it yields a reducing sugar, but it is not altered by digestion with saliva or pancreatic juice (see also below under carbohydrates).

It has been already stated that purified mucin (except of bile) yields a carbohydrate when heated with acids or stronger alkalis, and a considerable controversy has been carried on as to whether animal-gum is a carbohydrate which occurs in the tissues as a mere companion of the mucins or whether it is in all cases a product of their decomposition. The evidence at hand on this point is not conclusive, and for the present it may be said that, while mucin is often accompanied by animal-gum, the latter has by no means been proved to take its origin from the former. The whole subject requires further investigation.

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[1] Jernström (Swedish). See Abst. in Maly's *Bericht.* 1880, S. 34.
[2] Wälchli, *Jn. f. prakt. Chem.* N. F. Bd. XVII. (1878), S. 71.
[3] See Liebermann, *Biol. Centralb.* Bd. VII. (1887–88), S. 60.
[4] *Zt. f. physiol. Chem.* Bd. VI. (1881), S. 75; VIII. (1883), S. 122.
[5] *Ibid.* Bd. IX. S. 367.
[6] *Centralb. f. d. med. Wiss.* (1885), S. 369. Pflüger's *Arch.* Bde. XXXIX. (1886). S. 193; XL. S. 21.

Gelatin or Glutin.[1]

The ultimate fibrils of connective tissue and the organic matter of which bones are largely composed consist of a substance named in the first case 'collagen,' in the second 'ossein.' They are obtained either by digesting carefully cleansed tendons with trypsin, which dissolves up all the tissue-elements except the true collagenous (gelatiniferous) fibrils,[2] or by extracting bones with *dilute* acids in the cold, by means of which the inorganic salts are dissolved and the ossein remains as a swollen elastic mass which retains the shape of the original bone. As thus prepared they are insoluble in water, saline solutions, and either cold dilute acids or alkalis; in the former, however, (acids) they swell up to a transparent gelatinous mass. When subjected to prolonged boiling with water, more especially under pressure as in a Papin's digester, they are gradually dissolved, and the solution now contains true gelatin into which they have been converted by hydrolysis, and has acquired the characteristic property of solidifying into a jelly on cooling. The conversion of collagen into gelatin may be still more easily effected by a shorter boiling in presence of dilute acids, but in this case, unless the process be carefully regulated, the first-formed gelatin is further hydrolysed into what are often spoken of as gelatin-peptones. Although insoluble in dilute acids collagen is readily dissolved by digestion with pepsin in presence of an acid passing rapidly through the condition of gelatin into that of gelatin-peptone, and although collagen is not acted upon by trypsin in alkaline solution, it is readily hydrolysed by this enzyme after a short preliminary treatment with dilute acid or boiling water, the products as before being known as gelatin-peptones. When gelatin is exposed for some time in the *dry* condition to a temperature of 130° it is reconverted into a substance closely resembling collagen, which may be again converted into gelatin by treatment with water under pressure at 120°.[3]

Gelatin obtained by the above means from connective tissue or bones is, when dry, a transparent, more or less coloured and brittle substance.[4] It is insoluble in cold water, but swells up into an elastic flexible mass which now dissolves readily in water when warmed. When the solution is again cooled it solidifies characteristically into a jelly even when it contains as little as 1 p. c. of gelatin; it is also readily soluble in the cold in dilute acids and alkalis. The proteid reactions of gelatin are so feeble that they must be regarded as due entirely to unavoidably admixed traces of proteid impurities; more particularly is it to be noticed that the usual reaction of proteids with Millon's reagent is entirely wanting, a fact which indicates the probable absence of aromatic (benzol) residues in its molecule and corresponds to the absence of tyrosin among the products of its decomposition. Notwithstanding that it is in no sense a proteid, its percentage composition approximates to that of the latter class of substances and may be taken as $C=50·76$, $H=7·15$, $O=23·21$, $N=18·32$, from which it appears to contain distinctly less carbon than do the proteids; it is also stated to contain no sulphur when pure, but ordinarily it contains a small amount (·5 p. c.).[1] Gelatin is precipitated by but few salts, viz.: mercuric chloride and the double iodide of mercury and potassium in acid solution. Several acids on the other hand precipitate it readily, such as phosphotungstic and metaphosphoric, also taurocholic and tannic. Of the two last-named acids the former yields an opalescence in presence of 1 part of gelatin in 300,000 of solution, and the latter in still more dilute solutions.[2] The precipitability with tannic acid seems to depend on the presence of neutral salts.[3] The specific rotatory power of gelatin in aqueous solution or in presence of a trace of alkali is stated to be $(\alpha)_D = -130°$ at 30° C. and to be reduced to $-112°$ or $-114°$ on the addition of more alkali or acetic acid.[4] This statement requires confirming.

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[1] Glutin must not be confounded with the vegetable proteid 'gluten.'

[2] Kühne u. Ewald, *Verhand. d. naturhist.-med. Ver.* Heidelb. Bd. I. N.F. (1877), S. 3. See also Etzinger, *Zt. f. Biol.* Bd. x. (1874), S. 84. Ewald, *Ibid.* Bd. XXVI. (1889), S. 1.

[3] Hofmeister, *Zt. f. physiol. Chem.* Bd. II. (1878), S. 313. Weiske, *Ibid.* VII. (1883), S. 460.

[4] Pure gelatin is colourless, *e. g.* fine isinglass prepared from the bladder of the sturgeon. Glue is impure gelatin made from hides, &c.

When decomposed in seal tubes with caustic-baryta gelatin yields on the whole the same products as do the proteids,[5] with the exception of tyrosin; neither this nor any other substance of the typically aromatic series is ever obtained during any decomposition of gelatin, whether by chemical or putrefactive processes.[6] By prolonged boiling with hydrochloric acid it yields glycin (glycocoll), leucin, glutamic acid, and ammonia,[7] and with sulphuric acid aspartic acid as well.[8]

Gelatin-peptones.[9] By prolonged boiling with water (1 p.c. solution boiled for 30 hours), or shorter treatment in a Papin's digester, also by heating with hydrochloric acid (4 p.c. at 40°), or still more readily by pepsin in presence of acid or by trypsin,[1] gelatin loses its power of solidifying on cooling, and is converted into more highly soluble and now *diffusible* substances, to which the name of gelatin-peptones has been given. A similar change occurs when gelatin is taken into the stomach.[2] From the conditions under which the change is effected and from certain evidence deducible from analysis there can be but little doubt that the conversion takes place as the result of hydrolysis, as in the case of the formation of true peptones from proteids.

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[1] Hammarsten *Zt. f. physiol. Chem.* Bd. IX. (1885), S. 305.

[2] Emich *Monatshefte f. Chem.* Bd. VI. (1885), S. 95.

[3] Weiske, *loc. cit.*

[4] J. de Bary, *Diss.* Tübingen, 1864. Also in Hoppe-Seyler's *med.-chem. Unters.* Hft. 1, 1866, S. 73.

[5] Schützenberger et Bourgeois, *Compt. Rend.* T. LXXXII. (1876), p. 262.

[6] Nencki. See Abst. in Maly's *Bericht.* 1876, S. 31. Jeanneret, *Jn. f. prakt. Chem.* (N.F.) Bd. XV. (1877), S. 353. Weyl, *Zt. f. physiol. Chem.* Bd. I. (1877), S. 339.

[7] Horbaczewski, *Sitzb. d. Wien. Akad.* Bd. LXXX. (1879), 2 Abth. Juni.-Hft.

[8] Gaehtgens, *Zt. f. physiol. Chem.* Bd. I. (1877), S. 299.

[9] Hofmeister, *Zt. f. physiol. Chem.* Bd. II. (1878), S. 299. Gives literature down to that date. Tatarinoff, *Compt. Rend.* T. XCVII. (1883), p. 713.

Recent researches have shown that the hydrolytic decomposition of gelatin by digestive enzymes gives rise to products analogous to those obtainable by the same method from the proteids. Thus during both its peptic and tryptic digestion certain primary products are formed to which the name gelatoses or glutoses may be applied, and which have so far been distinguished as proto- and deutero-gelatose. Accompanying these, in variable amount, are other products known as gelatin-peptones. The latter are to be regarded as a product of the further action of the enzymes on the first formed gelatoses and, like the true peptones in their relationship to the albumoses, may be separated from them by their non-precipitability on saturation with ammonium sulphate, a reagent which completely precipitates the gelatoses. Protogelatose is partially precipitated by saturation of its solution with common salt, and completely so on the simultaneous addition of acetic acid. Deuterogelatose is not precipitated by either of the above reagents.[3] The so-called true gelatin-peptones have not yet been obtained in sufficient quantity to admit of their complete examination. The products of the digestion of gelatin appear to give a distinct biuret reaction with caustic soda and sulphate of copper, and like the peptones (and albumoses) are not precipitated by taurocholic acid, which precipitates gelatin from its solutions.[4]

When the spores of Penicillium are sown on a surface of gelatin, as soon as the mycelium is well developed the subjacent gelatin liquefies sometimes to a considerable depth, so that the Penicillium finally floats on a layer of fluid separated by some distance from the remaining still solid gelatin. The fluid in this layer now yields an intense biuret reaction. A similar liquefaction is observed during the growth of certain bacteria and other micro-organisms on gelatin.

The fact has already been referred to (§ 524) that gelatin taken as food, while it materially lessens both the nitrogenous, and to some slight extent the non-nitrogenous metabolism of the body, and thus appears able to undergo a destructive metabolism similar to that of the proteids, cannot, on the other hand, play any part in the constructive nitrogenous metabolism which leads to the formation of proteids. In other words the nitrogen contained in gelatin cannot be built up into the nitrogen of a proteid.[1] We do not as yet possess any information which enables us to formulate any reason for this special behaviour of gelatin. It has been suggested that the absence of aromatic residues in gelatin (see above) might account for the phenomenon,[2] but experiments in which animals have been fed with gelatin+tyrosin have not confirmed this view.[3] It appears that a considerable amount of gelatin is digested and absorbed in man, since none appears in the faeces, and meat (muscle) may contain as much as 2 p. c. of gelatin: further, Voit's experiments show that a dog may digest and absorb 50 p.c. of the gelatin administered in the form of bones.[4] Bearing these facts in mind and knowing that gelatin appears to be more readily metabolised than proteids, we may regard gelatin as a valuable food-stuff, but not as a food which can supply the nitrogenous needs of the tissues themselves. The facts thus stated may supply an explanation of the beneficial effects which are supposed to result from the use of jellies in training diets.[5]

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[1] Schweder, *Inaug.-Diss.* Berlin, 1867.

[2] Uffelmann, *Arch. f. klin. Med.* Bd. xx. (1877), S. 535.

[3] Chittenden and Solley, *Jl. of Physiol.* Vol. xii. (1891), p. 23. See also Klug, Pflüger's *Arch.* Bd. xlviii. (1890), S. 100. The latter author describes further a product to which he gives the name apoglutin. It makes its appearance as an insoluble substance, hence resembling antialbumid or dyspeptone, during the digestion of gelatin.

[4] Emich, *Monatshefte f. Chem.* Bd. vi. (1885), S. 95.

Chondrin.

The matrix of hyaline cartilage is composed of an elastic, semi-transparent substance which is insoluble in cold or hot water and does not swell up appreciably by treatment with either water or dilute acetic acid. By prolonged treatment with water under pressure in a Papin's digester it is gradually dissolved and yields a solution which gelatinises on cooling and now contains the substance usually spoken of as chondrin. The hyaline matrix of cartilage appears thus to bear the same relationship to chondrin that the ground-substance of connective-tissue (collagen) does to gelatin, and is therefore frequently spoken of as 'chondrigen.'

The substance known as chondrin, which is obtained in solution by the action of superheated water on hyaline cartilage, exhibits the following characteristic reactions.[6] It is precipitated by acetic acid, which does not, even if in considerable excess, redissolve the precipitate; minute quantities of mineral acids similarly cause a precipitate to appear which is in this case readily soluble in the slightest excess of the acids. These reactions suffice to distinguish between chondrin and gelatin, and a further distinction may be made on the basis of the fact that solutions of chondrin are precipitated by several reagents such as alum, normal lead acetate, and other metallic salts (of Ag and Cu), which yield no precipitate with gelatin, while on the other hand mercuric chloride and tannin do not precipitate chondrin but are characteristic precipitants of gelatin (see above). Chondrin is powerfully laevorotatory; in faintly alkaline solution $(\alpha)_D = -213{\cdot}5°$; in presence of excess of alkali this becomes $(\alpha)_D = -55{\cdot}20°$.[1]

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[1] Voit, *Zt. f. Biol.* Bd. VIII. (1872), S. 297; x. (1874), S. 203.

[2] Hermann u. Escher, *Vierteljahrsch. d. natforsch. Gesell. in Zurich*, 1876, S. 36.

[3] Lehmann, *Sitzber. d. Gesell. f. Morphol. u. Physiol.* München, 1885.

[4] See also Etzinger, *Zt. f. Biol.* Bd. x. (1874), S. 84.

[5] For a statement of the nutritional, metabolic and physiological significance of gelatin see Hermann's *Hdbch. d. Physiol.* Bd. VI. Sn. 123, 318, 391, 395.

[6] Moleschott u. Fubini, Moleschott's *Untersuch.* Bd. XI. (1872), S. 104.

By prolonged treatment with boiling water, or shorter heating with dilute (1 p.c.) sulphuric acid or stronger hydrochloric acid, chondrin is decomposed with the formation of a nitrogenous crystallisable product which characteristically reduces alkaline solutions of cupric oxide.[2] Opinions however differ considerably as to the real nature of this reducing substance. It was at one time regarded as a true carbohydrate, and more recently Landwehr has identified it with his animal-gum.[3] (See above *sub* mucin.) There is now but little doubt that it contains nitrogen, is possessed of distinct acid properties, and exhibits marked carbohydrate affinities apart from its reducing powers.[4] According to the older and some recent observers its solutions are laevorotatory,[5] but v. Mering states that it is dextrorotatory.[6] Its real nature cannot be regarded as yet as definitely established. When the action of the boiling acids is prolonged, or if caustic alkalis or barium hydrate is employed, chondrin undergoes a further profound decomposition resulting in the formation of a large number of crystalline products; with regard to these the fact of chief importance and interest is the general presence among them of leucin, and the entire absence of tyrosin and glycin (glycocoll), and the occurrence of aspartic and glutamic acids in very minute traces only, if at all.[7]

We have so far spoken of chondrin as a distinct and individual substance; the view has however been put forward that it is in reality merely a mixture of mucin and gelatin,[8] and the outcome of more recent work seems to be tending towards the strengthening of this view.[9] When hyaline cartilage is extracted with baryta water or dilute alkalis a solution is obtained which yields reactions typical of the so-called chondrin and closely resembling those characteristic of mucin; the undissolved residue when boiled with water is dissolved into a solution which gives the reactions in general typical of gelatin. Mörner, treating sections of hyaline cartilage in succession with dilute hydrochloric acid (·1—·2 p.c.) and caustic potash (·1 p.c.), finds that rounded masses of the matrix are dissolved out and leave thus a residual network. The dissolved parts consist largely of a substance (chondromucoid) with marked affinities to mucin, whereas the undissolved network, by treatment with acids or superheated water, is converted largely into typical gelatin. For further details the original papers already quoted should be consulted.

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[1] de Bary, *loc. cit.* (sub gelatin).

[2] v. Mering, *Inaug.-Diss.* Strassburg, 1873.

[3] Pflüger's *Arch.* Bd. XXXIX (1886), S. 198.

[4] Krukenberg, *Zt. f. Biol.* Bd. XX. (1884), S. 307. Mörner (Swedish). See abst. in Maly's *Bericht.* 1887, S. 308; 1888, S. 217.

[5] Petri, *Ber. d. deutsch. chem. Gesell.* Jahrg. XII. (1879), S. 267.

[6] See Hoppe-Seyler's *Hdbch. d. physiol.-path. chem. Anal.* (5 Auf. 1883), S. 301.

[7] Schützenberger et Bourgeois, *cit.* (sub gelatin).

[8] Morochowetz, *Verhand. d. naturhist.-med. Ver.* Heidelbg. Bd. I. (1876), Hft. 5.

[9] Krukenberg, Mörner, *loc. cit.*

Elastin.

This is the characteristic component of the elastic fibres which remain after the removal of gelatin, mucin, fats, etc., from tissues such as "ligamentum nuchae." Some of the more important ways in which it differs from the substances which have been previously described are sufficiently stated by describing the method of its preparation in a pure form.[1] Ligamentum nuchae of an ox is cut into fine slices, treated for three or four days with boiling water, then for some hours with 1 p.c. caustic potash at 100°C and subsequently with water. This process is then repeated with 10 p.c. acetic acid. Finally it is treated for 24 hours in the cold with 5 p.c. hydrochloric acid, washed with water, boiled with 95 p.c. alcohol, and extracted for at least two weeks with ether to remove every trace of adherent fat. By the above method it may be obtained as a pale yellowish powder in which the shape of fragments of the original elastic fibres may be still distinguished under the microscope. When moist it is yellow and elastic, but on drying it becomes brittle and may with difficulty be pulverised in a mortar. Sulphur probably does not enter into its composition (?). It may be dissolved by strong alkalis at 100°C, and it also goes into solution when treated with mineral acids at the same temperature; but in the latter case the solution involves decomposition with the formation of much leucin (30—40 p.c.) and traces (·25 p.c.) of tyrosin when the acid employed is sulphuric.[2] If strong hydrochloric acid be employed with chloride of zinc the same crystalline products are obtained together with ammonia, glycocoll, and an amidovalerianic acid, but no glutamic or aspartic acids.[3] In this respect it differs from both ordinary proteids and gelatin, since the former when similarly treated yield the glutamic and aspartic acids but no glycocoll, and the latter never yields the least trace of tyrosin. During the putrefactive decomposition of elastin products similar to the above are obtained together with some peptone-like substance.[4] When treated with superheated water, or with dilute hydrochloric acid at 100°C. or with pepsin or trypsin in acid and alkaline medium respectively, elastin is more or less rapidly dissolved and undergoes a true digestive change, during which products are formed many of whose general reactions are analogous to those of the digestive products of proteids.[1] It is however as yet uncertain whether a true elastinpeptone can be obtained; it is more probable that during the digestion only some of the primary substances (elastoses) make their appearance, since they are completely precipitated by saturation with neutral ammonium sulphate.[2] Elastin is also rapidly corroded and dissolved by the action of papaïn. (Gamgee.)

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[1] Horbaczewski, *Zt. f. physiol. Chem.* Bd. VI. (1882), S. 330. Chittenden and Hart, *Zt. f. Biol.* Bd. XXV. (1889), S. 368.
[2] Erlenmeyer u. Schöffer, *Jn. f. prakt. Chem.* Bd. LXXX. (1860), S. 357.
[3] Horbaczewski, *Monatshefte f. Chem.* Bd. VI. (1885), S. 639
[4] Wälchli, *Jn. f. prakt. Chem.* (N.F.), Bd. XVII. (1878), S. 71.

Hilger[3] has obtained a somewhat similar substance from the shell and yolk of certain snakes' eggs.

Keratin.

Hair, nails, feathers, horn, and the epidermal structures in general are composed chiefly of keratin, admixed however with small quantities of proteids and other substances, from which it may be freed by thorough extraction with water, alcohol, ether, and dilute acids in succession, followed by digestion with pepsin and trypsin (Kühne) and a renewed washing with the above reagents. A convenient source which readily yields a pure product, owing to the comparatively simple composition of the mother substance, is found in the shell-membrane of ordinary eggs.[4] The percentage composition of keratin is in general allied to that of the true proteids, but varies within somewhat wide limits according to the source from which it has been prepared and particularly with regard to the sulphur which it contains This latter element varies in amount from ·5 to 5·0 p.c. and leads to the formation of sulphides of the metal when keratin is dissolved in alkalis. Unlike the proteids, gelatin and elastin, keratin is quite unaffected by the most prolonged and active digestion with either pepsin or trypsin. On the other hand, when decomposed at high temperatures by either caustic baryta or strong hydrochloric acid, it yields large quantities of leucin (15 p.c.), tyrosin (3—4 p.c.) and other products which are in general identical with those obtained by the similar treatment of proteids.[5] It is soluble in strong alkalis when heated, and is further stated to be dissolved by prolonged treatment with superheated water; in the latter case a product is obtained to which, since it somewhat resembles an albumose, the name keratinose has been given, and which may now be digested by means of pepsin.[6] Further investigation in this direction is however needed before any positive statements can be made respecting any truly digestive products derivable from keratin, or indeed as to the characteristic differences of the keratins from different sources.

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[1] Horbaczewski, *loc. cit.*

[2] Chittenden and Hart, *loc. cit.*

[3] *Ber. d. deutsch. chem. Gesell.* 1873, S. 166. See also Krukenberg, *Vergl.-physiol. Stud.* II. R. 1. Abth. S. 68.

[4] Lindwall (Swedish). See abst. in Maly's *Jahresber.* 1881, S. 38.

[5] Horbaczewski, *Sitzb. d. k. Akad. d. Wiss. Wien.* Bd. LXXX. (1879), 2 Abth. Juni-Hft. Blennard, *Compt. Rend.* T. LXXXIX. (1879), p 953, T XC. (1880), p. 612.

[6] Krukenberg, *Sitzb. d. Jena, Gesell. f. Med. u. Nat.-wiss.* 1886, S. 22.

Lindwall (*loc. cit.*) described the formation of an albuminate and a peptone-like (? albumose) substance during the treatment of keratin with dilute (1 — 2 p.c.) caustic soda at digestion temperatures.

Neurokeratin.[1]

When the substance of the brain or any mass of medullated nerves is thoroughly extracted with water, alcohol, and ether, and then digested with pepsin and trypsin in succession, a residue is obtained which closely resembles the ordinary keratins, and constitutes about 15—20 p.c. of the whole brain after it has been freed from its medullary constituents by alcohol and ether.[2] This residue is neurokeratin, so named from the source from which it is obtained. It is characterised by its somewhat greater resistance to those decomposing agents whose action on keratin has been already described. The determination of its existence in tissues which are not obviously epidermal in the adult is of considerable embryological and morphological interest, since it throws some light upon the developmental origin of the structures in which it is present or absent.[3]

Chitin. $C_{15}H_{26}N_2O_{10}$.[4]

Although it is not found as a constituent of any mammalian tissue, this substance composes the chief part of the exoskeleton of many invertebrates. It is by many regarded as the animal analogue of cellulose of plants, and from this point of view it possesses considerable morphological interest. The most convenient source from which it may be prepared is the cleansed exoskeleton of crabs or lobsters. This is first thoroughly extracted with dilute hydrochloric acid and caustic potash, after which it is treated with boiling alcohol and ether, and may be finally completely decolorised by the action of permanganate of potash.[5] It is a white amorphous substance, which often retains the shape of the integument from which it has been prepared. It is insoluble in any reagents other than concentrated mineral acids, such as sulphuric or hydrochloric. The immediate addition of water to these solutions probably reprecipitates the chitin in an unaltered form.[6] When heated with concentrated hydrochloric acid it is decomposed into glycosamin and acetic acid, of which the former is the characteristic product.[1] A similar decomposition is observed when sulphuric acid is employed.

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[1] Kühne u Ewald, *Verhand. naturhist.-med. Ver.* Heidelbg. Bd. 1, 1877, S. 457. Kühne u. Chittenden, *Zt. f. Biol.* Bd. xxvi. (1890), S. 291.

[2] See also Chevalier, *Zt. f. physiol. Chem.* Bd. x. (1886), S. 100.

[3] Cf. Smith, H. E., *Zt. f. Biol.* Bd. xix. (1883), S. 469. Steinbrügge, *Ibid.* Bd. xxi. (1885), S. 631.

[4] Ledderhose, *Zt. f. physiol Chem.* Bd. ii. (1878), S. 213. But see also Sundwik, *Ibid.* Bd. v. (1881), S. 384.

[5] Bütschli, *Arch. f. Anat. u. Physiol.* Jahrg. 1874, S. 362.

[6] But see Hoppe-Seyler, *Hdbch. d. physiol.-path. Anal.* 5 Aufl. 1883, S. 188. Krukenberg, *Zt. f. Biol.* Bd. xxii. (1886), S. 480

Glycosamin ($C_6H_{13}NO_5$). Crystallises from alcohol in fine needles, is dextrorotatory, and reduces Fehling's fluid to the same extent as does dextrose, but is not fermentable. By treatment with nitrous acid a carbohydrate ($C_6H_{12}O_6$) (?) is obtained which also reduces cupric oxide, but is similarly unfermentable. This is doubtless the substance which led to certain erroneous statements as to the production of a true dextrose from chitin.[2]

Nuclein. $C_{29}H_{49}N_9P_3O_{22}$ (?).

The nuclei of cells, both animal and vegetable, differ distinctly in chemical composition from the remaining substance of the cells. As a result of this difference it is possible to separate the nuclei approximately by various means from the adjacent cell-substance. The name nuclein is given to the material of which the nuclei or parts of nuclei thus isolated chiefly consist. When, however, the statements of the various authors who have dealt with nuclein are compared with regard to the reactions, decompositions, and more especially the percentage composition of their preparations, it appears probable that no definite substance exists to which the one name nuclein may be fitly applied. It may be that the discrepancies are due to the existence of several kinds of nuclein;[3] but this is as yet scarcely proved, and it is on the whole more probable that the different results of the various authors must be attributed to the impurity of the substance on which they operated.[4] In accordance with this view it is to be observed that the percentage of phosphorus obtained in even the most reliable analyses is stated to vary from 2·3 to 9·6 p.c.

After the above precautionary remarks we may now give an account of the preparation and properties of the so-called nuclein. When a mass of cells such as pus,[5] yeast,[6] nucleated red blood-corpuscles,[7] salmon-milt,[8] or egg-yolk[9] is extracted with water and dilute (·5 p.c.) hydrochloric acid, the cells are largely broken up and dissolved, and the nuclei separated from them. A further purification is obtained by treatment with alcohol and ether and final digestion with pepsin in acid solution, which does not affect the substance of the nuclei.[1] The final residue thus obtained is washed with dilute acid, dissolved in very weak caustic soda, precipitated by hydrochloric acid, and washed with water and alcohol. Prepared by the above methods, nuclein is an amorphous substance, rich in phosphorus, which is set free as phosphoric acid when it is boiled with alkalis. At the same time some form of proteid usually makes its appearance, as also do the crystalline substances of the xanthin series, guanin (?) and hypoxanthin, when the nuclein is heated with dilute mineral acids instead of alkalis.[2] It appears, however, that the absolute and relative amount of the above possible products of its decomposition varies with the source from which the nuclein is obtained.

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[1] Ledderhose, *loc. cit.* and *Ibid.* Bd. IV. (1880), S. 139.

[2] Berthelot, *Compt. Rend.* T. XLVII. (1858), p. 227. *Journ. de la Physiol.* T. II. p. 577.

[3] Hoppe-Seyler, *Hdbch. d. physiol.-path. chem. Anal.* (5 Auf.), 1883, S. 303. *Physiol. Chem.* S. 85.

[4] Worm-Müller, Pflüger's *Arch.* Bd. VIII. (1874), S. 190. Bunge, *Physiol.-pathol. Chem.* (Transl. by Wooldridge, 1890), p. 89

[5] Miescher, Hoppe-Seyler's *Med.-chem. Untersuch.* Hft. IV. (1871), S. 452. Hoppe-Seyler, *Ibid.* S. 486.

[6] Hoppe-Seyler, *Ibid.* S. 500. Kossel, *Zt. physiol. Chem.* Bd. III. (1879), S. 284; IV. (1880), S. 290; VII. (1883), S. 7. *Unters. üb. d. Nucleine u. ihre Spaltungsprod.* Strassb. 1881. Loew, Pflüger's *Arch.* Bd. XXII. (1880), S. 62.

[7] Brunton, *Jl. Anat. and Physiol.* 2 Ser. Vol. III. 1869, p. 91. Plósz, Hoppe-Seyler's *Med.-chem. Unters.* Hft. IV. (1871), S. 461.

[8] Miescher, *Verhand. d. Natforsch. Gesell.* Basel, Bd. VI. (1874), S. 138.

[9] Miescher, Hoppe-Seyler's *Med.-chem. Unters.* Hft. IV. (1871), S. 502. Worm-Müller, *loc. cit.*

Under the name 'adenin' Kossel has more recently described a new base which he obtained by the decomposition of nuclein from yeast-cells with dilute sulphuric acid and heat.[3] It is crystalline, readily soluble in warm water and caustic alkalis, and when treated with nitrous acid yields hypoxanthin. (See below.)

$$C_5H_5N_5+H_2O=C_5H_4N_4O+NH_3.$$

When egg- or serum-albumin is precipitated with metaphosphoric acid, a phosphorised substance is obtained which exhibits many of the reactions characteristic of nuclein.[4] It does not, however, yield any of the xanthin bases when treated with acids.[5]

Nucleo-albumins.

While the nuclei may be regarded as composed principally of the somewhat unsatisfactorily characterised nucleins, there is evidence of the existence[6] of closely allied substances to which, since they appear to be a compound of nuclein and a proteid, the name nucleo-albumin has been given. Our knowledge of these substances is as yet rudimentary and imperfect, and subsequent investigation must decide their real nature and their relationship to the nucleins.

The more characteristic reactions of the nucleo-albumins may be stated as follows. Soluble in very dilute alkalis, they are readily reprecipitated by acetic acid; and the constancy in properties of the product obtained by repeated solution and precipitation seems to show that they are not mere mixtures of nuclein and proteid. Their behaviour towards alkalis and acetic acid is such as to lead to an easy confusion with the mucins. When digested with pepsin they yield peptones and albumoses, and a phosphorised residue which is in most respects identical with nuclein, but does not appear to yield products of the xanthin series when decomposed by acids. They are, like the globulins, precipitated from solution by neutral salts, — the precipitate becoming swollen and slimy when the precipitant is sodium chloride or magnesium sulphate, but not so when sodium sulphate is employed.

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[1] It also resists the action of trypsin. Bókay, *Zt. f. physiol. Chem.* Bd. I. (1877), S. 157.

[2] Kossel, *loc. cit.* Also *Zt. f. physiol. Chem.* Bd. V. (1881), Sn. 152, 267; Bd. VIII. (1884), S. 404.

[3] *Ber. d. d. chem. Gesell.* 1885, Sn. 79, 1928. *Zt. physiol. Chem.* Bd. X. (1886), S. 250. Schindler, *Ibid.* Bd. XIII. (1889), S. 432. Bruhns, *Ibid.* Bd. XIV. (1890), S. 533.

[4] Liebermann, *Ber. d. d. chem. Gesell.* (1888), S. 598. Pflüger's *Arch.* Bd. XLVII. (1890), S. 155.

[5] Pohl, *Zt. f. physiol. Chem.* Bd. XIII. (1889), S. 292.

[6] Worm-Müller, Pflüger's *Arch.* Bd. VIII. (1874), S. 194.

It is impossible as yet to give any general method of separating the nucleo-albumins from the parent protoplasm. Reference to the works quoted below is essential when dealing with any investigation as to their presence in particular cases.

When casein is digested with pepsin a residue of nuclein is left; and it appears probable that casein may be in reality a compound of this substance with a proteid, or that it is a nucleo-albumin.[1] Egg-yolk is also considered by some authors to contain nuclein as a nucleo-albumin, which is further stated to be ferruginous,[2] but by others the yolk is spoken of as yielding only nuclein. Whichever view be correct, the nuclein of yolk does not yield members of the xanthin series by decomposition with acids,[3] — resembling in this respect the nuclein from milk. Synovial fluid[4] and bile (?)[5] are also stated to contain substances which, though resembling mucin in physical properties, are probably nucleo-albumins.

It may be pointed out that in some of the above cases the nucleo-albumin is obtained from non-nuclear sources. When, on the other hand, aqueous extracts are made of certain nucleated structures, there is evidence that apart from the nuclein of the nuclei, some nucleo-albumin is obtained whose presence is referred rather to the cell-protoplasm than to the nuclei: this is the case with liver-cells,[6] the cells of the submaxillary gland,[7] and lymph-corpuscles.[8] Non-nucleated red blood-corpuscles do not yield any nucleo-albumin.[9]

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[1] Lubavin, Hoppe-Seyler's *Med.-chem. Unters.* Hf. IV. (1871), S. 463. See also *Ber. d. deutsch. chem. Gesell.* 1877, S. 2238. Hammarsten, *Zt. f. physiol. chem.* Bd. VII. (1883), S. 273.

[2] Bunge, *Zt. f. physiol. Chem.* Bd. IX. (1885), S. 49. See also his Text-book, p. 100.

[3] Kossel, *Arch. f. Physiol.* Jahrg. 1885, S. 346.

[4] Hammarsten (Swedish). See Abst. in Maly's *Ber.* Bd. XII. (1882), S. 480.

[5] Paijkull, *Zt. f. physiol. Chem.* Bd. XII. (1888), S. 196.

[6] Plósz, Plüger's *Arch.* Bd. VII. (1873), S. 371. Hammarsten, *Ibid.* Bd. XXXVI. (1885), S. 351.

[7] Hammarsten, *Zt. f. physiol. Chem.* Bd. XII. (1888), S. 174.

[8] Halliburton, *Jl. of Physiol.* Vol. IX. (1888), p. 235.

[9] Halliburton and Friend, *Ibid.* Vol. X. (1889), p. 543.

CARBOHYDRATES.[1]

Certain members only of this extensive class have been found in the human body; of these, the most important and wide-spread are glycogen, grape-sugar or dextrose (glucose), with which diabetic sugar seems to be identical,[2] maltose, and milk-sugar. Inosit, which has the same percentage composition as a sugar ($C_6H_{12}O_6$) and possesses a distinctly sweet taste, has hence been usually classed with the carbohydrates. This is incorrect, since it is now known to belong to the benzol series (see below, p. 108).

Although the above-mentioned carbohydrates may be detected in various tissues and secretions of the animal body, their presence in the several cases is not so much due to their introduction into the body in the form in which they there occur as to their production from other members of the carbohydrate group existing in food. The chief of these is starch, and it will perhaps conduce to completeness to deal first very briefly with this parent-substance and some of the products of its decomposition.

THE STARCH GROUP.

1. **Starch** $(C_6H_{10}O_5)_n$.

Starch occurs characteristically in plants and is formed in their green parts under the determinant influence of the chlorophyll-corpuscles. The exact mode of its formation is however as yet undecided. It exists in plant-tissues in the form of grains which vary in size and shape according to the plant, but which possess the common characteristic of exhibiting a stratified structure, which is much more marked in some cases (potato-starch) than in others, and the phenomena of double-refraction when examined in polarised light. Considered as a whole the grains appear to be composed of two substances of which the chief both in quantity and importance is called granulose and the other cellulose. The former, which yields the blue colour characteristic of starch on the addition of iodine, may be dissolved out by the action of saliva or malt-extract, leaving a cellulosic skeleton of the original grain. This so-called cellulose is not identical with ordinary cellulose, as shown by its ready solubility in several reagents which do not dissolve the latter.[3] When treated with boiling water the grains swell up and finally burst, yielding a uniform viscous mass of starch-paste of which the chief component is the granulose. The mass thus obtained cannot be regarded as a true solution of starch, and it filters with extraordinary difficulty, leaving a gelatinous residue on the filter, however dilute the starch-paste may be which is used for the filtration. When subjected to hydrolytic agencies such as superheated water, dilute acids and enzymes the starch passes rapidly into true solution, yielding at the same time a series of successive products to be described below.

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[1] The carbohydrates are very fully treated in Tollens' *Hdbch. d. Kohlenhydrate*, Breslau, 1888. See also Miller's *Chemistry*, Pt. III. Sec. 1 (1880), p. 567 *et seq.*

[2] There is perhaps some slight doubt as to this identity, based chiefly upon a slight apparent difference in the specific rotatory power of true dextrose and that obtained from diabetic urine. (See Miller's *Chemistry*, p. 583.)

[3] Brown and Heron, *Jl. Ch. Soc.* Vol. XXXV. (1879), p. 611. Liebig's *Ann.* Bd. CXCIX. S. 165.

Many attempts have been made to assign a definite formula to this substance. The outcome of these is that the molecule of starch is certainly not $C_6H_{10}O_5$ but n $(C_6H_{10}O_5)$, where n is not less than 5 or 6 and is probably much larger.

When starch is converted into dextrose by treatment with dilute boiling sulphuric acid, it is found that 99 parts of starch yield 108 of dextrose.[1] Thus

$$[(C_6H_{10}O_5)_6 + H_2O] \text{ (mol.} = 990) + 5H_2O = 6C_6H_{12}O_6 \text{ (mol.} = 1080).$$

Most recently, and in continuation of previous researches, it has been shown, by an application of Raoult's method, that the molecule of soluble starch must probably be represented by the formula $5\ (C_{12}H_{20}O_{10})_{20}$.[2] Formulae based on analyses of the supposed compound of starch with iodine are probably valueless, since there is but little reason to suppose that any such definite compound exists.

2. **Soluble starch** (Amylodextrin) $(C_6H_{10}O_5)_n$.

When starch-paste, heated to 40° C. on a water-bath, is digested with a small amount of saliva and the whole stirred so as to effect a thorough mixture of the two, the paste rapidly loses its opalescent appearance, becoming limpid and clear like water: the moment this change has taken place the digesting mixture should be boiled to cut short the further action of the ptyalin. The fluid thus obtained contains the first product of the hydrolysis of starch to which the name of 'soluble starch' has been given. Its solution filters readily, and the filtrate yields with iodine the pure blue characteristic of the original unaltered starch. On the addition of an excess of alcohol the soluble-starch is precipitated, the precipitate after drying being but little soluble in cold water although it readily dissolves in water at 60—70° C. It also yields a characteristic precipitate with tannic acid, and differs in this respect from the dextrins.[3] It is dextrorotatory

$$(\alpha)_D = +194{\cdot}8° \ [(\alpha)_j = 216°],$$

and does not reduce Fehling's fluid. The same substance may be similarly obtained by the limited action of malt-extract or pancreatic juice.

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[1] Sachsse, *Sitzb. d. Natforsch. Gesell.* Leipzig, 1877. *Chem. Centralb.* 1877, No. 46.

[2] Brown and Morris, *Jl. Chem. Soc.* Vol. LV. July, 1889, p. 462.

[3] Griessmayer, *Annal. d. Chem.* Bd. CLX. (1871), S. 40.

3. **The dextrins** $(C_6H_{10}O_5)_n$.[1]

When the hydrolytic action of saliva, malt-extract, or pancreatic juice on starch-paste is prolonged, the first-formed soluble-starch is rapidly changed into a number of successive substances to which the general name of dextrin is given. These products are intermediate between soluble-starch and the sugars which result from the complete hydrolysis of starch, and are probably very numerous, the similarity in the properties of the successively formed dextrins rendering their separation and characterisation extremely difficult. They are all precipitable by alcohol, and differ from soluble-starch in yielding no precipitate with tannic acid.

(i) *Erythrodextrin.* If during the earlier stages of the hydrolysis of starch-paste, successive portions of the solution be tested by the addition of iodine, it may be observed that the pure blue which it yields at first passes gradually through violet and reddish-violet to reddish-brown, the latter colour being supposedly due to the presence in the solution of erythrodextrin, whence the name. But little is definitely known of this dextrin as a chemical individual, its chief characteristic being the colour it yields with iodine.[2] The violet observed during the earlier stages of hydrolysis is due to an admixture of the blue due to soluble-starch with the red of the erythrodextrin.

Commercial dextrin, which is very impure, containing dextrose and frequently unaltered starch, usually yields a very strong red colouration of the addition of iodine.

(ii) *Achroodextrin.*[3] When, during the *prolonged* enzymic hydrolysis of starch under ordinary conditions, the addition of iodine ceases to give any colouration, the fluid now contains much sugar together with a considerable but variable proportion of this dextrin, which has received its name from its behaviour towards iodine, yielding no colour with this reagent. It is the characteristic dextrin obtained during the *prolonged* artificial digestion of starch with saliva (or pancreatic juice) and may be separated from its solution by concentration and the addition of an excess of alcohol. As thus prepared it is mixed with much adherent maltose (see below), from which it cannot be entirely freed by washing with alcohol or by successive solution in water and reprecipitation with alcohol. A partial separation may be obtained by fermenting off the sugar with yeast (O'Sullivan) or by dialysis, since dextrin is non-diffusible. If however the mixture be warmed with a slight excess of mercuric cyanide and caustic soda, the whole of the sugar is destroyed in reducing the mercuric salt, leaving in solution a non-reducing dextrin.[1] As thus prepared it appears to possess a constant dextrorotatory power $(\alpha)_D = 194{\cdot}8°$ $[(\alpha)_j = 216°]$, and as precipitated by alcohol is a white amorphous powder very soluble in water.

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[1] For the probable value of n in certain cases, see Brown and Morris, *cit. sub* starch.

[2] But see Musculus u. Meyer, *Zt. physiol. Chem.* Bd. IV. (1880), S. 451.

[3] Brown and Morris, *Jl. Ch. Soc.* Vol. XLVII. (1885), p. 551.

Maltodextrin.[2] This substance is described as appearing during the earlier stages of a limited hydrolysis of starch-paste with diastase, and it may perhaps similarly occur when saliva or pancreatic juice is employed. It differs from the dextrins previously described as follows. It is more soluble in alcohol and distinctly diffusible; it reduces Fehling's fluid, has a lower specific rotatory power

$$(\alpha)_D = +174{\cdot}2° \; [(\alpha)_j = 193{\cdot}1°],$$

and is completely convertible into maltose by the further action of diastase. It will therefore not be found among the products of a prolonged hydrolytic degradation of starch.

When starch-paste is hydrolysed outside the body with diastase or with animal enzymes some dextrin is always obtained together with the sugars which make their characteristic appearance during the process. Considerable difference of opinion has been expressed as to the possibility of a complete conversion of these dextrins into sugar by the renewed action of the enzyme upon them after their isolation, but the balance of opinion appears to be that the conversion is in many cases either impossible or takes place with slowness and difficulty. If this is so then the course of an artificial and normal digestion of starch is, as regards the final products, very different in the two cases, for there is no evidence that in the body any carbohydrate is absorbed as dextrin from the alimentary canal. The conditions however under which the two digestions are carried on are markedly different, and more particularly with respect to the very complete and continuous removal of digestive products in the natural process as compared with their accumulation in an ordinary artificial digestion. Now there is no doubt that the products of an enzymic hydrolysis are inhibitory to the further action of the enzyme,[3] and this is probably the cause of the observed difference. In accordance with this, if a starch digestion be carried on in an efficient dialyser, the starch may be practically entirely converted into sugar, the small residue of dextrin being due rather to inefficiency of the apparatus than to the chemical resistance of the dextrins to complete conversion into sugar.[1] Although this statement is based upon experiments made with saliva, there is no reason to suppose that the same will not hold good in the case of the pancreatic juice by whose action the chief carbohydrate digestion of the body is carried on. We shall therefore not be far wrong in concluding that in the animal body starch is completely converted into sugar previous to absorption, and if this be the case the interest of the physiologist in the primary products of starch hydrolysis becomes very small, except so far as a study of these products is essential to the elucidation of the probable molecular magnitude and structure of the parent-substance.

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[1] It should be carefully borne in mind that probably many forms of dextrin exist, especially among the earlier products of hydrolysis, none of which give any colouration with iodine.

[2] Brown and Morris, *loc. cit.* p. 561.

[3] See also Lindet, *Compt. Rend.* T. CVIII. (1889), p. 453, with special reference to maltose.

When starch is treated with dilute boiling acids, the products which have been so far described are formed in rapid succession, the whole being finally converted into dextrose.[2]

4. **Animal-gum** ($C_{12}H_{20}O_{10}+2H_2O$) (?).

This is, according to Landwehr, a form of carbohydrate which may be extracted by the prolonged action of superheated water from salivary and mucous glands, and is found also in milk and urine. It has already been briefly described above (p. 79), where its chief characteristics have been given. To these may here be added that it yields no colouration with iodine, is very feebly dextrorotatory and appears to form a compound with cupric oxide; the latter is obtained when caustic soda and sulphate of copper are added to its solution, and may be used for the separation of animal-gum from urine.[3]

5. **Glycogen** ($(C_6H_{10}O_5)_n$).

This substance is from a purely chemical point of view extremely like starch, the similarity being most marked when the hydrolytic products of the two are compared. A study of its occurrence, behaviour, and fate in the animal body leaves but little doubt that it may be regarded from the physiological side as truly the animal analogue of the vegetable starch, and as such it is frequently spoken of as 'animal starch.' It was first described as a constituent of the liver by Bernard[4] and, simultaneously though independently, by Hensen.[5] In more recent times it has been found to occur in greater or less quantities in many tissues of the adult body, as for instance the muscles,[1] also in white blood- and pus-corpuscles[2] and other contractile protoplasm (Aethalium septicum),[3] in which its presence is significantly connected with their specialised activity, not as an essential, as some have supposed, but as a convenient accessory. It is also conspicuously found in the tissues of the embryo before the liver is functionally active,[4] and is present in large quantities in many mollusks, as for instance the common oyster[5] (9·5 p.c.).

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[1] Lea, *Jl. of Physiol.* Vol. XI. (1890), p. 226.

[2] But see Wohl, *Ber. d. d. chem. Gesell.*, Jahrg. XXIII. (1890), S. 2101.

[3] Landwehr, *Centralb. f. d. Med. Wiss.*, 1885, S. 369. See also Wedenski, *Zt. f. physiol. Chem.* Bd. XIII. (1889), S. 122.

[4] *Gaz. méd. de Paris*, 1857, No. 13. *Compt. Rend.* T. XLIV. (1857), p. 579. *Gaz. Hebdom.* 1857, No. 28.

[5] *Arch. f. path. Anat. u. Physiol.* Bd. XI. (1857), S. 395.

It is at present uncertain whether the glycogen obtainable from muscles is identical with that of the liver. It is stated that muscle-glycogen yields a distinctly more purple colour with iodine than does liver glycogen,[6] but their identity is still an open question.[7]

Preparation of glycogen. The liver of an animal (rabbit or dog), previously fed with copious meals of carbohydrate, is excised as rapidly as possible, cut into small pieces, and thrown into an excess of boiling water, at least 400 c.c. to each 100 gr. of liver. After being boiled for a short time, the pieces are removed, ground up as finely as possible in a mortar with sand or powdered glass, returned to the original water, and boiled again for some time. On faintly acidulating the boiling mass with acetic acid a large amount of the proteid matter in solution is coagulated and may be removed by filtration. The filtrate is now rapidly cooled, and the proteids finally and completely precipitated by the alternating addition of hydrochloric acid and of a solution of the double iodide of mercury and potassium (Brücke's reagent),[8] as long as any precipitate is formed. The precipitated proteids are again removed by filtration, the glycogen precipitated by the addition of two volumes of 95 p.c. alcohol,[9] collected on a filter, washed thoroughly with 60 p.c. spirit, and finally with absolute alcohol and ether (Brücke).[10]

The above method suffices in cases where there is much glycogen present and no quantitative result is desired ; as a matter of fact there is a not inconsiderable loss during its application. The accurate determination of glycogen in tissues is a matter of some difficulty, primarily because it is not easy to ensure the complete separation into solution of the glycogen from the tissue, and secondarily owing to a possible loss during the precipitation and removal of the proteids with which it is always largely contaminated. The first difficulty may be largely overcome by the addition of caustic potash which dissolves the tissue fragments and thus liberates the glycogen; also by extraction in a Papin's digester,[1] in which case the solution is again very complete.[2]

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[1] Nasse, Pflüger's *Arch.* Bd. II. (1869), S. 97.

[2] Hoppe-Seyler, *Med.-chem. Unters.* Hft. 4 (1871), S. 486.

[3] See refs. on p. 4. Also Kühne, *Physiol. Chem.* 1868, S. 334.

[4] See Preyer's *Specialle Physiol. d. Embryo*, Leipzig, 1885, S. 271.

[5] Bizio, *Compt. Rend.* T. LXII. (1866), p. 675.

[6] Naunyn, *Arch. f. exp. Path. u. Pharm.* Bd. III. (1875), S. 97. Boehm u. Hoffmann, *Ibid.* Bd. x. (1878), S. 12. Nasse, Pflüger's *Arch.* Bd. XIV. (1877), S. 479.

[7] See also Musculus u. v. Mering, *Zt. f. physiol. Chem.* Bd. II. (1878), S. 417.

[8] Prepared by saturating a boiling 10 p.c. solution of potassium iodide with freshly precipitated iodide of mercury; on cooling, th. [illegible] is filtered and the filtrate employed as directed.

[9] So that the mixture contains 60 p.c. of alcohol.

[10] *Sitzb. d. Wien. Akad.* Bd. LXIII. (1871), 2 Abth. Feb.-Hft., S. 214.

Glycogen is, when pure, an amorphus white powder, readily soluble in water with which it yields a solution which is usually, but not always, opalescent. This solution contains no particles which are visible under the microscope and filters readily without diminution of the opalescence; the latter may be largely removed by the addition of free alkalis or acetic acid. Under ordinary conditions it is readily precipitated by alcohol when the mixture contains 60 p.c. of the precipitant, but if pure, and in 0·5—1·0 p.c. solution, even an excess of absolute alcohol is stated not to cause its precipitation. The precipitation takes place at once on the addition of a trace of sodium chloride.[3]

It gives a characteristic port-wine colouration with iodine, which does not however distinguish it from erythrodextrin since in both cases the colour, contrary to the older and current statements, disappears on warming and returns on cooling. On the other hand, dextrins are not precipitated by 60 p.c. alcohol, even the most insoluble of these substances requiring at least 85 p.c. of alcohol for their precipitation, and usually more. It appears that the reaction with iodine is most delicate in presence of sodium chloride.[4]

Aqueous solutions of glycogen are strongly dextrorotatory, but the statements as to its specific rotatory power must be received with caution. [*Boehm and Hoffmann*[5] $(a)_D = +226{\cdot}7°$. *Külz*[6] in ·6 p.c. solution $(a)_D = +203{\cdot}5°$ to $+225{\cdot}6°$. *Landwehr*[7] $(a)_D = +213{\cdot}3°$].

The molecular magnitude of glycogen, like that of starch, is unknown. Glycogen yields precipitates with tannic acid, also with calcium and barium hydrate,[8] and with basic lead acetate. No reliance can however be placed on the determination of the molecular weight of glycogen from an analysis of these compounds.

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[1] Boehm, Pflüger's *Arch.* Bd. XXIII. (1880), S. 44.

[2] The whole subject is very fully treated by Külz in *Zt. f. Biol.* Bd. XXII. (1886), S. 161, where also the literature is comprehensively quoted. See additionally Nasse, Pflüger's *Arch.* Bd. XXXVII. (1885), S. 582, and Landwehr, *Ibid.* XXXVIII. S. 321. Panormow (Polish). See Abst. Maly's *Jahresb.* 1887, S. 304. Cramer, *Zt. f. Biol.* Bd. XXIV. (1888), S. 67.

[3] Külz, *Ber. d. d. chem. Gesell.* Jahrg. 1882, S. 1300.

[4] Nasse, Pflüger's *Arch.* Bd. XXXVII. (1885), S. 585.

[5] *Arch. f. exp. Path. u. Pharm.* Bd. VII. (1877), S. 489.

[6] Pflüger's *Arch.* Bd. XXIV. (1881), S. 85.

[7] *Zt. f. physiol. Chem.* Bd. VIII. (1883), S. 170.

[8] Nasse, Pflüger's *Arch.* Bd. XXXVII. (1885), S. 582.

The hydrolytic products obtained by the action of enzymes and dilute boiling acids on glycogen have not been as fully studied as they have in the case of starch, but the general course of the decomposition is the same in both cases. Thus when treated with dilute mineral acids at 100°C., the opalescence disappears, some dextrin is formed *en passant,* and finally the solution contains only dextrose.[1] On the addition of saliva or pancreatic juice to a solution of glycogen at 40°, the first change observed is an immediate disappearance of the opalescence, followed by a rapid conversion into some form of dextrin and a considerable proportion of a sugar which is apparently identical with maltose.[2] Some trace of dextrose may perhaps at the same time be formed.

The change which glycogen in the liver undergoes post-mortem and presumably also during life is strikingly different from that which has been described above. Whereas by ordinary enzymic hydrolysis, maltose is the chief final product obtained, there is now no doubt that in the liver little if any maltose is formed, the so-called liver-sugar being apparently identical with true dextrose. This fact throws considerable light on the mode of conversion of glycogen into sugar by the liver. It has been most usually taught that this conversion is due to some fermentative action; if this were so then the enzyme which is the active agent must be possessed of powers differing from those of most other enzymes since it forms dextrose and not maltose. But as a matter of fact it does not appear possible to extract any appreciable quantity of enzyme from the liver, and if a trace is obtained it is of one whose action on starch and glycogen yields chiefly maltose and not dextrose. It is hence a legitimate conclusion that the conversion of glycogen into sugar by the liver is the outcome of the specific metabolic activity of the hepatic cells, and not of any enzymic action.[3] It is also significantly probable, from what has been already said (see above, p. 59), that the liver receives its carbohydrates supplied in the form of dextrose, and there is no doubt that diabetic sugar is closely related to, if not identical with, true dextrose.

The dextrin which some observers have obtained from muscles is not to be regarded as a specific constituent, but as formed from their glycogen by some post-mortem change. Horse-flesh is peculiarly rich in glycogen, and it was chiefly from this source that dextrin was obtained in large amount.[4]

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[1] Maydl, *Zt. f. physiol. Chem.* Bd. III. (1879), S. 194. Külz u. Bornträger, Pflüger's *Arch.* Bd. XXIV. (1881), S. 28. Seegen, *Ibid.* Bd. XIX. (1879), S. 106.

[2] Musculus u. v. Mering, *Zt. f. physiol. Chem.* Bd. II. (1878), S. 403. Seegen, *loc. cit.* Külz, Pflüger's *Arch.* Bd. XXIV. (1881), S. 81.

[3] Eves, *Jl. of Physiol.* Vol. V. (1884), p. 342 (contains lit. to date). See more recently Langendorff, *Arch. f. Physiol.* 1886. Suppl.-Bd. S. 277. Panormow, *Klin. Wochenb.* 1887, No. 27. Dastre, *Arch. de Physiol.* (4) T. I. (1888), p. 69.

[4] Limpricht, Liebig's *Ann.* Bd. CXXXIII. (1865), S. 293.

6. **Cellulose** $(C_6H_{10}O_5)_n$.

Although true cellulose is never found as a constituent of the animal tissues, it possesses no inconsiderable interest for the physiologist in view of the fact that in the herbivora a large amount of cellulose is digested and absorbed so that it does not reappear externally in the excreta. In man also there is no doubt that some digestion and absorption of cellulose may occur, the process being facilitated by the fact that in those more succulent vegetables and fruits in which it is taken by man, the cell-walls are comparatively non-lignified and hence more easily acted upon by the digestive agents.

The lignification of the cell-wall which has taken place in those plant tissues to which the name 'woody' is ordinarily applied is due to the presence of a substance called lignin. Very little is known of it as a chemical individual: it appears to contain more carbon than does cellulose. Its discrimination from cellulose depends on the fact that it is coloured yellow by the action of Schulze's reagent (see below) and deep brown by that of iodine and sulphuric acid. When treated with phloroglucin and strong hydrochloric acid it turns red; it is coloured bright yellow by the action of aniline sulphate or chloride and the subsequent addition of hydrochloric acid.

Further, although at present but little is known as to how the digestion of cellulose is brought about in the alimentary canal, there is increasing evidence of the possible existence of a specific enzyme to whose solvent action the change is due. But as yet this evidence rests almost entirely upon experiments with and observations of vegetable organisms.[1]

Cellulose is related to starch and in some cases (Date, Phytelephas) plays the part of a store of reserve material, being dissolved, presumably by some enzyme, and utilised during germination. The cell-wall of vegetable cells is composed of cellulose, which in young cells is pure and much less resistant to various reagents than it is in the older cells where it has become lignified and incrusted with other substances. When pure it is soluble in one reagent only, viz. Schweizer's which is a solution of hydrated cupric oxide in ammonia.[2] When treated with strong sulphuric acid cellulose is changed and yields a substance which is coloured blue by iodine; a similar colouration is observed on the addition of iodine after the action of chloride of zinc (Schulze's reagent).[1] These reactions afford a means of detecting cellulose.

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[1] Brown and Morris, *Jl. Chem. Soc.* Vol. LVII. (1890), p. 497. Contains references to other literature.

[2] Prepared as follows. Sulphate of copper in solution, to which some ammonium chloride has been added, is precipitated with caustic soda: the hydrated cupric oxide thus obtained is washed, and dissolved to saturation in 20 p.c. ammonia. It may also be prepared by pouring strong ammonia on to copper turnings, the requisite oxidation of the copper being effected by drawing a current of air through the fluid in which the turnings are immersed. (Cross and Bevan, *Cellulose*, 1885, p. 6.)

By treatment with strong sulphuric acid cellulose may be dissolved with the formation of a dextrin-like product: on diluting with water and boiling it is finally converted into a sugar which is apparently identical with dextrose.[2]

As already stated cellulose is undoubtedly digested in the alimentary canal more especially of herbivora, but also to a less extent of man.[3] We know however but little of the real nature of the digestive processes which are involved in this. Two views are open to us. It has long been known that under the influence of putrefactive organisms, as from sewer-slime, cellulose is disintegrated and dissolved with an evolution of marsh-gas and carbonic anhydride.[4] This is usually known as the marsh-gas fermentation of cellulose. In accordance with this it is possible that a similar factor is at work in the alimentary canal, more especially of the herbivora with their large cæcum in which the food stays for some time. This accords with the marked occurrence of marsh-gas in the gases of their intestine and its increased presence in the intestine of man when largely fed with a vegetable diet.[5] On the other hand it is possible that the digestion may turn out to be due to some definite enzyme,[6] but as yet no such enzyme has been obtained with certainty from the secretions or tissues of the alimentary canal. Possibly the organisms which as stated above can cause the decomposition of cellulose do so by means of some specific enzyme. It remains for further research to throw a decisive light on the possibilities to which attention has been drawn.

Some difference of opinion exists as to the physiological significance of cellulose digestion. There is at present no evidence that the cellulose of food as such is a food-stuff in the same sense that starch is. As far as the existing evidence goes we shall not perhaps be far wrong in supposing that cellulose digestion is primarily important as liberating from the cells the true food-stuffs which they contain. At the same time the products formed during the solution of the cellulose may, if they are oxidised in the body, contribute to its energy and thus be of use.[1]

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[1] The reagent used is prepared as follows. Iodine is dissolved to saturation in a solution of chloride of zinc, sp.gr. 1·8, to which 6 parts of potassium iodide have been added. See also Bower, *Pract. Bot.*, 1891, p. 506. Cross and Bevan (*loc. cit.* p. 7) recommend the following. Zinc is dissolved to saturation in hydrochloric acid, and the solution evaporated to sp.gr. 2·0; to 90 parts of this solution are added 6 parts of potassium iodide dissolved in 10 parts of water, and in this solution iodine is finally dissolved to saturation.

[2] Flechsig, *Zt. f. Physiol. Chem.* Bd. VII. (1883), S. 523.

[3] Bunge, *Physiol. and Path. Chem.* 1890, pp. 81, 191.

[4] Popoff, Pflüger's *Arch.* Bd. X. (1875), S. 113. Van Tieghem, *Compt. Rend.* T. LXXXVIII. (1879), p. 205. Hoppe-Seyler, *Ber. d. d. chem. Gesell.* Jahrg. XVI. (1883), S. 122. *Zt. f. physiol. Ch.* Bd. X. (1886), Sn. 201, 401.

[5] Tappeiner, *Ber. d. d. chem. Gesell.* Jahrg. XV. (1882), S. 999; XVI. Sn. 1734, 1740. *Zt. f. Biol.* Bd. XX. (1884), S. 52. (Gives literature to date.) *Ibid.* S. 215; XXIV. (1888), S. 105.

[6] Hofmeister, *Arch. f. Thierheilk.* Bd. VII. (1881), S. 169; XI. (1885), Hfte. 1, 2.

7. **Tunicin** $(C_6H_{10}O_5)_n$.

This substance constitutes the chief part of the mantle of Tunicata (Ascidians) and appears to have been first described by C. Schmidt,[2] who drew attention to its similarity to vegetable cellulose. This view was confirmed by Berthelot, who however observed that it is much more resistant to the action of acids than is true cellulose.[3] In other respects the two may be regarded as identical. In accordance with this it is found that tunicin is soluble in Schweizer's reagent (see above), from which it may be reprecipitated by hydrochloric acid and thus purified. It is further coloured blue by the addition of iodine after preliminary treatment with sulphuric acid. It is soluble in concentrated sulphuric acid, and if water be added to this solution and it be boiled for some time, a sugar which is apparently identical with ordinary dextrose is obtained.[4]

It is prepared in the pure form by treating the mantles for some days with water in a Papin's digester, then in succession with boiling dilute hydrochloric acid, strong caustic potash and water. As thus obtained it retains the form of the parent tissue.

THE SUGARS.

The researches of Emil Fischer have thrown a flood of light on the chemistry of the sugars.[5] In phenyl-hydrazin $(C_6H_5.NH.NH_2)$ he discovered a reagent which forms with the sugars compounds known as hydrazones and osazones. These provided for the first time by their various solubilities, melting-points, and rotatory powers an adequate means of detecting, separating, and characterising the several members of this class of carbohydrates. Hence it became possible to investigate the occurrence of sugars among the complicated products of the reactions employed in the effort to effect their transformations and synthetic production. It would be out of place here to enter into the details of Fischer's work, and it must suffice to say that he has not merely synthetised both dextrose and lævulose, and definitely established the fact that they are respectively an aldehyde and ketone of the hexacid alcohol $C_6H_8(OH)_6$, but has in addition succeeded in producing artificial sugars containing seven, eight, and nine carbon atoms.[1] In connection with the latter an interesting question arises as to the probable effects on animal metabolism of their introduction into the body instead of the natural sugars.

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[1] On the above see Weiske, *Chem. Centralb.* Bd. XV. (1884), S. 385. Henneberg u. Stohmann, *Zt. f. Biol.* Bd. XXI. (1885), S. 613. Weiske (Ref.) Schulze u. Flechsig, *Ibid.* XXII. S. 373.

[2] Liebig's *Ann.* Bd. LIV. (1845), S. 318.

[3] *Ann. d. Ch. et Phys.* 3 Sér. T. LVI. (1859), p. 149.

[4] Franchimont, *Ber. d. d. chem. Gesell.* 1879, S. 1938. *Compt. Rend.* T. LXXXIX. (1879), p. 755. Schäfer, Liebig's *Ann.* Bd. CLX. (1871), S. 312.

[5] Fischer has given a condensed account of his researches, with full references to the literature, in *Ber. d. d. chem. Gesell.* Jahrg. XXIII. (1890), S. 2114. Of this an abstract is given in *Jl. Chem. Soc.* Nov. 1890, p. 1223. See also Schulz, *Biol. Centralb.* Bd. X. (1890), Sn. 551, 620.

The osazones. The compounds of the sugars to which this generic name is applied are formed when solutions of the sugars are warmed for some time on a water-bath with phenyl-hydrazin and dilute acetic acid, and separate out either in an amorphous or crystalline state. Their formation takes place in two stages. In the first the sugar combines, as do the aldehydes and ketones, with one molecule of the base to form a compound which is in most cases readily soluble and is known as a hydrazone. In the second stage the first-formed hydrazone is oxidised by the excess of phenylhydrazin present, and the substance thus produced unites with another molecule of the base to form the osazone. As already stated the osazones of the various sugars differ characteristically in their solubilities, melting-points, and rotatory powers. They hence afford an invaluable means not only for detecting and isolating the sugars, but also for discriminating between sugars whose optical and reducing powers may afford an insufficient distinction. Further, in some cases the osazones have provided a means of ascertaining the molecular formula of certain sugars and of determining the constitution of others. The characteristic properties of the several osazones are given below under the respective sugars.

THE DEXTROSE GROUP.

1. **Dextrose** (Glucose, Grape-sugar).

$C_6H_{12}O_6$. $[COH-(CH.OH)_4-CH_2.OH]$.

Is found in minute but fairly constant quantities as a normal constituent of blood, lymph, and chyle. Its occurrence in the liver has been already referred to (§ 465) in connection with diabetes, a disease which is characterised by the large amount of dextrose which is present in the blood and the still larger amount in the urine. The question whether dextrose is a normal constituent of urine has led to much dispute, but it now appears probable that it is present in minute amounts.[2] The experimental difficulties of detecting traces of sugar in this excretion are considerable. There is no dextrose normally in bile.

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[1] Fischer u. Passmore, *Ber. d. d. chem. Gesell.* Jahrg. XXIII. (1890), S. 2226.

[2] For literature and results see Neubauer u. Vogel, *Analyse des Harns* (Ed. IX. 1890), S. 41.

The probability that it is as dextrose that the carbohydrates are finally absorbed from the alimentary canal has already been referred to (p. 59). This corresponds with the fact that dextrose is the most readily assimilable sugar, as is known from comparative injections of the various sugars into the blood-vessels and observations on their subsequent appearance in the urine.

When pure, dextrose is colourless and crystallises from its aqueous solution in six-sided tables or prisms, often agglomerated into warty lumps. The crystals will dissolve in their own weight of cold water, requiring however some time for the process; they are very readily soluble in hot water. Dextrose is somewhat sparingly soluble in cold ethyl-alcohol, more soluble in warm; slowly soluble, but in considerable quantity, in methyl-alcohol, and insoluble in ether.

It may be prepared by concentrating diabetic urine until it yields crystals of dextrose; these are then purified by recrystallisation from methyl-alcohol. It may also be conveniently prepared by the action of hydrochloric acid on cane-sugar dissolved in alcohol.[1] A freshly prepared cold aqueous solution of dextrose possesses a specific rotatory power for monochromatic yellow light of $(a)_D = + 100°$. This rapidly falls, especially on warming, until it may be taken as $(a)_D = + 52·5°$ for solutions which do not contain more than 10 p.c. of the sugar. The rotation is however dependent on the concentration of the solution being least with very dilute solutions.

The specific rotatory power of a substance is the amount, measured in degrees, by which the plane of polarised light is rotated by a solution which contains 1 gram of the substance in each 1 c.c. when examined in a layer 1 dcm. in thickness. Since the amount of rotation produced in any given case is directly proportional to the specific rotatory power, also to the weight of substance in solution and the thickness of the fluid layer in which it is examined we have $a = (a) \times p \times l$ or $(a) = \frac{a}{p \, . \, l}$, where (a) is the specific rotatory power, p is the weight in grams of the substance in 1 c.c. of the solution, l is the thickness in decimetres of the fluid layer and a is the observed rotation. This equation provides a means of estimating sugars quantitatively by measuring the rotation produced by a solution of unknown concentration in a layer of known thickness, the specific rotatory power being known.[2]

The instruments employed for measuring the amount of rotation produced by an optically active substance are known generically as Polarimeters. In one class of these instruments the source of light used is a brightly luminous sodium-flame, the determination being made for the monochromatic light corresponding to the D line of the solar spectrum. In this case the specific rotatory power is represented by $(\alpha)_D$. In another class the mean yellow light of an argand or paraffin lamp is employed. In this form of polarimeter the field of the instrument when adjusted for use is of a pale pinkish-violet colour, called from the extreme sensitiveness with which it changes from pink to violet or the reverse the 'transition tint' (teinte de passage). This colour is complementary to yellow (jaune), and specific rotatory powers determined for this particular colour are represented by $(a)_j$. For any given substance $(\alpha)_D$ is always less than $(\alpha)_j$, and for ordinary purposes $(\alpha)_D = \frac{(\alpha)_j}{1{\cdot}108}$, or $(\alpha)_D : (\alpha)_j :: 21{\cdot}65 : 24$. Hence it is important in all cases to state clearly whether a given determination has been made for monochromatic yellow light or for the 'transition tint' of mean yellow light.

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[1] Soxhlet, *Jn. f. prakt. Chem.* (N.F.) Bd. XXI. (1880), S. 227.

[2] For details of the instruments and methods see Landolt, *Das optische Drehungsvermögen organ. Substanzen.* Hoppe-Seyler, *Physiol. path. chem. Anal.* 1883, S. 24. Miller's *Chem.* (Ed. by Armstrong and Groves), Pt. III. 1880, p. 569 et seq.

Dextrose, like all alcohols, readily forms compounds with acids and many salts; of these the latter are the more important and are in many cases characteristic, as for instance those with caustic alkalis and sodium chloride. When heated many of these compounds, more particularly those of bismuth, copper, and mercury, are decomposed, the decomposition being accompanied by the precipitation either of the metal (Hg) or of an oxide (Cu_2O). This fact provides the basis for the more important methods of detecting the presence of dextrose and other sugars with similar reducing powers, and of estimating them quantitatively in solution, since it is found that the amount of reduction effected by any given sugar is, under given conditions, a constant quantity.[1]

Phenyl-glucosazone. $C_{18}H_{22}N_4O_4$. $[C_6H_{10}O_4\ (C_6H_5.N_2H)_2]$.

This compound of dextrose with phenyl-hydrazin crystallises in yellow needles. It is almost insoluble in water, very slightly soluble in hot alcohol, melts at about 205°, and is lævo-rotatory when dissolved in glacial acetic acid. The phenyl-hydrazin test for dextrose is applied as follows. To 50 c.c. of the suspected fluid (e.g. diabetic urine) add 1—2 grm. hydrochloride of phenyl-hydrazin, 2 grm. sodium acetate, and heat on a water-bath for half an hour; or else add 10—20 drops of pure phenyl-hydrazin and an equal number of drops of 50 p.c. acetic acid and warm as before.[2] On cooling, if not before, the glucosazone separates out as a crystalline or it may be amorphous precipitate. If amorphous it is dissolved in hot alcohol, the solution is then diluted with water and boiled to expel the alcohol, whereupon the compound is obtained in the characteristic form of yellow needles. By the above method it is possible to obtain the crystals from fluids which contain only 0·5 grm. per litre.

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[1] The description of the various methods employed for the detection and estimation of dextrose and other sugars lies outside the scope of this work. Full details are given in Neubauer u. Vogel, *Analyse des Harns*, and Tollens' *Handbuch der Kohlenhydrate*.

[2] Fischer, *Ber. d. d. chem. Gesell.* Bd. XXII. (1889), S. 90 (foot-note).

An important property of dextrose is its power of undergoing fermentations. Of these the principal are: (1) *Alcoholic.* This is produced in aqueous solutions of dextrose, under the influence of yeast. The decomposition is the following: $C_6H_{12}O_6 = 2C_2H_6O + 2CO_2$, yielding (ethyl) alcohol and carbonic anhydride. Higher alcohols of the fatty series are found in traces, as also are glycerin, succinic acid, and probably many other bodies. The fermentation is most active at about 25°C. Below 5°C. or above 45°C. it almost entirely ceases. If the saccharine solution contains more than 15 per cent. of sugar it will not all be decomposed, as excess of alcohol stops the reaction. (2) *Lactic.* This is best known as occurring in milk when it turns sour owing to the conversion of lactose into lactic acid. But dextrose and other sugars may also be converted into lactic acid ($C_6H_{12}O_6 = 2C_3H_6O_3$), the conversion being ordinarily due to the presence of some specific micro-organism[1] which is specially active in presence of decomposing nitrogenous material, such as decaying cheese.[2] A similar change is rapidly produced when dextrose is mixed with finely divided gastric mucous membrane.[3] There is also some evidence of the existence of an unorganised ferment (enzyme) in the mucous membrane of the stomach which can convert lactose and dextrose (?) into lactic acid.[4] On prolonged standing the lactic fermentation is apt to pass into (3) *Butyric.* This results from the appearance and action of another specific organised ferment on the first formed lactic acid, the change being accompanied by the evolution of hydrogen and carbonic anhydride —

$$2C_3H_6O_3 = C_3H_7 . COOH. + 2CO_2 + 2H_2.$$

Lactic and butyric fermentations are most active at 35° and 40° respectively; they probably occur constantly in the alimentary canal with a carbohydrate diet and may in some cases be remarkably predominant. The hydrogen evolved during butyric fermentation probably plays some important part in the production of the fœcal and urinary pigments from those of bile (see below).

Dextrose is the sugar which is characteristically formed by the action of boiling dilute mineral acids on sugars of the cane-sugar group, and on starch and dextrin. When it is dissolved in concentrated sulphuric acid it is said to be partly reconverted into a true dextrin which may be precipitated by the addition of alcohol, and is capable of reconversion into dextrose by mineral acids.[1]

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[1] Lister, *Path. Soc. Trans.* 1873, p. 425. *Quart. Jl. Micros. Sci.* Vol. XVIII. (1878), p. 177. Marpmann, *Centralb. f. allg. Gesundheitspfl.* Ergänzungshft. II. (1886), S. 117. Meyer, *Inaug.-Diss.* Dorpat, 1880. Abst. in Maly's *Bericht.* 1881, S. 468.

[2] Bensch, *Preparation of lactic acid.* Liebig's *Ann.* Bd. LXI. (1847), S. 174.

[3] Maly, Liebig's *Ann.* Bd. CLXXIII. (1874), S. 227.

[4] Hammarsten (Swedish). See Abst. in Maly's *Ber.* Bd. II. (1872), S. 118.

2. **Lævulose.**

$C_6H_{12}O_6$. $[CH_2.OH - CO - (CH.OH)_3 - CH_2.OH]$.

This is the ketone corresponding to the aldehyde dextrose. It is best known as occurring mixed with dextrose in many fruits, also in honey, and is stated to occur occasionally in urine. It is a characteristic product of the action of dilute mineral acids on cane-sugar, which is hereby decomposed into equal parts of dextrose and lævulose, and since when the change is complete the original dextro-rotatory power of the solution has become lævo-rotatory, the cane-sugar is said to have been 'inverted.' A similar inversion takes place in the stomach and small intestine (see under cane-sugar). In its general reactions lævulose behaves like dextrose, but may be at once distinguished from the latter by its powerful lævo-rotatory action on polarised light: this varies considerably with the temperature and concentration of the solution. It yields with phenyl-hydrazin an osazone identical with that derived from dextrose. It forms a compound with calcium hydrate which unlike that yielded by dextrose is extremely insoluble and may thus be employed for the separation of the two sugars.

2. **Galactose (Cerebrose)** $C_6H_{12}O_6$.

When milk sugar (lactose), see p. 113, is boiled with dilute mineral acids it is decomposed into a molecule of dextrose and one of galactose

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6.$$

The two sugars may be separated by crystallisation and by taking advantage of the greater solubility of galactose in absolute alcohol.[2] In its general reactions and behaviour galactose resembles dextrose but is possessed of a considerably greater specific rotatory power $[(\alpha)_D = +83°]$ which increases with the concentration and rise of temperature.[3] It yields with phenyl-hydrazin an osazone (phenyl-galactosazone) which has the same composition as phenyl-glucosazone and very similar solubilities. It differs however from the latter in melting at 190—193° and in being optically inactive when dissolved in glacial acetic acid. It has recently been shown that the sugar which was described by Thudichum[4] as resulting from the action of boiling dilute sulphuric acid on certain constituents of the brain substance, and was named by him cerebrose, is really identical with galactose.[1]

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[1] Musculus u. Meyer, *Zt. f. physiol. Chem.* Bd. v. (1881), S. 122.

[2] Fudakowski, *Ber. d. d. chem. Gesell.* Jahrg. 1875, S. 599. Soxhlet, *Jn. f. pr. Chem.* (2) Bd. xxi. (1880), S. 269.

[3] Meissl, *Jn. f. pr. Chem.* Bd. xxii. (1880), S. 97.

[4] *Jn. f. pr. Chem.* Bd. xxv. (1882), S. 19.

Galactose is fermentible with yeast, but less readily so than is dextrose.

4. **Glycuronic acid.** $C_6H_{10}O_7$. [COH – (CH . OH)$_4$ – COOH].

This acid was first obtained as a compound, campho-glycuronic acid, in the urine of dogs after the administration of camphor,[2] and subsequently as urochloralic acid after the administration of chloral.[3] Since then it has been found in urine as ethereal or glucose-like compounds, with an extensive series of members of the fatty or aromatic series after the introduction of the appropriate substances into the animal body.[4] It is probable that traces of compounds of this acid occur normally in urine, since this excretion is usually slightly lævo-rotatory, and it is known that indol and skatol which are formed in the alimentary canal readily reappear in the urine as compounds of glycuronic acid; viz. indoxyl- and skatoxyl-glycuronic acid, when introduced into the body. The compounds of glycuronic acid are all lævo-rotatory, and some of them reduce metallic salts on boiling, and may hence lead to errors in the determination of sugar in urine.

Glycuronic acid does not occur in the free state in the animal body. Chemically it is closely related to dextrose; when oxidised with bromine it yields saccharic acid,[5] $C_6H_{10}O_8$, [COOH – (CH . OH)$_4$ – COOH], — an acid which is also readily obtained by the oxidation of dextrose with nitric acid. Saccharic acid can be converted into glycuronic acid by reduction with sodium amalgam.[6] Like dextrose, glycuronic acid is dextro-rotatory, but to a less extent, $(\alpha)_D = + 19{\cdot}4°$, reduces Fehling's fluid to the same extent as does dextrose, and forms with phenyl-hydrazin a yellow crystalline compound which melts at 114—115°. The acid is known only as a syrup soluble in alcohol and water. When boiled in the latter solvent it loses a molecule of water and yields an anhydride (lactone), $C_6H_8O_6$, which is crystalline, insoluble in alcohol, soluble in water, dextro-rotatory, and reduces Fehling's fluid powerfully.

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[1] Thierfelder, *Zt. f. physiol. Chem.* Bd. XIV. (1889), S. 209. Brown and Morris, *Jl. Chem. Soc.* Vol. LVII. (1890), p. 57.

[2] Schmiedeberg u. Meyer, *Zt. f. physiol. Chem.* Bd. III. (1879), S. 422.

[3] v. Mering, *Ibid.* Bd. VI. (1882), S. 480.

[4] For very full list of the various substances which when introduced into the body reappear in the urine as paired compounds with glycuronic acid, and for references to date (1890) to the literature of the subject, see Neubauer u. Vogel, *Harnanalyse*, Ed. IX. 1890, p. 116.

[5] Thierfelder, *Zt. f. physiol. Chem.* Bd. XI. (1887), S. 388. See also Bd. XIII. (1889), S. 275.

[6] Fischer u. Piloty, *Ber. d. d. chem. Gesell.* Jahrg. XXIV. (1891), S. 521.

The formation of the compounds of glycuronic acid, to which attention has been drawn, is of great and increasing interest. There can be little doubt that the acid has its origin in the carbohydrate (dextrose) of the body, but it is not yet possible to explain exactly how each particular compound arises after the introduction of the corresponding substance into the animal organism.[1]

Inosit. $C_6H_{12}O_6 + 2H_2O$. $[CH.OH]_6$.

This substance has the same percentage composition as a sugar, and possesses a distinctly sweet taste; in virtue of which properties it appears to have been usually classed with the carbohydrates. It does not, however, yield any of the reactions most typical of this class of substances; for instance, it exerts no rotatory power on polarised light, does not reduce metallic salts, does not undergo alcoholic fermentation, and does not react with phenyl-hydrazin. On account of these peculiarities, the view was long ago expressed that it is not a carbohydrate at all; and this has recently been shown to be the case by Maquenne, who has proved that it belongs really to the benzol series.[2] Structurally it may be represented by a closed ring of six CH . OH groups.

Inosit occurs but sparingly in the human body; it was found originally by Scherer[3] in the muscles. Cloetta showed its presence in the lungs, kidneys, spleen, and liver,[4] and Müller in the brain.[5] It occurs also in diabetic urine, and in that of 'Bright's disease,' and is found in abundance in the vegetable kingdom,—more especially in unripe beans, from which it may be conveniently prepared.[6] It is also found in the urine after the ingestion of an excess of water into the body.[7]

It is prepared from aqueous extracts of the mother tissues by acidulating with acetic acid and boiling to remove any coagulable proteids. The filtrate from these is then precipitated with normal lead acetate and filtered, and the inosit is finally precipitated from this filtrate by means of *basic* lead acetate in presence of ammonia. The lead compound is decomposed with sulphuretted hydrogen, and after the addition of alcohol and ether to the solution, inosit separates out by crystallisation.[8]

Pure inosit forms large efflorescent crystals (rhombic tables);

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[1] In the case of camphor and chloral see Fischer u. Piloty, *loc. cit.* S. 524.

[2] *Compt. Rend.* T. CIV. (1887), pp. 225, 297, 1719.

[3] *Ann. d. Chem. u. Pharm.* Bd. LXXIII. (1850), S. 322.

[4] *Ibid.* Bd. XCIX. S. 289.

[5] *Ibid.* Bd. CIII. S. 140.

[6] Vohl, *Ibid.* Bd. XCIX. (1856), S. 125; CI. S. 50.

[7] Külz, *Centralb. f. d. med. Wiss.* 1875, S. 933.

[8] Marmé, *Ann. d. Ch. u. Pharm.* Bd. CXXIX. S. 222. See also Boedeker, *Ibid.* Bd. CXVII. S. 118.

in microscopic preparations it is usually obtained in tufted lumps of fine crystals.

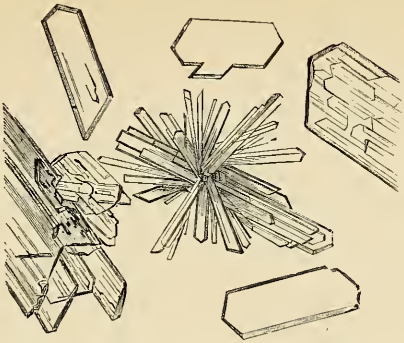

FIG. 1. INOSIT CRYSTALS. (After Kühne.)

Readily soluble in water, it is only slightly so in dilute alcohol, and is insoluble in absolute alcohol and ether.

Although inosit admits of no direct alcoholic fermentation, it has been stated to be capable of undergoing a lactic fermentation in presence of decomposing proteid (cheese) and chalk, yielding ordinary (ethylidene-) lactic acid and some butyric acid.[1] It had been previously stated that the acid thus obtained is sarcolactic (ethylene- or para-) lactic acid.[2] These assertions are scarcely reconcilable with our present knowledge of the chemical constitution of inosit.

Reactions of inosit.

(i) *Scherer's test.*[3] The suspected substance is treated with strong nitric acid and evaporated nearly to dryness on porcelain. On the addition of a little ammonia and a few drops of freshly prepared and not too dilute solution of calcium chloride, a bright pink or rose-coloured residue is obtained on renewed evaporation if inosit is present.

(ii) *Gallois' test.* When inosit in concentrated solution is treated with a few drops of 2 p.c. mercuric nitrate solution, or Liebig's solution for the estimation of urea, and the mixture is evaporated to dryness, it yields a yellow residue which on being more strongly heated turns rosy red; this disappears on cooling, and returns again on renewed heating.[4]

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[1] Vohl, *Ber. d. d. chem. Gesell.* Jahrg. 1876, S. 984.

[2] Hilger, *Ann. d. Chem. u. Pharm.* Bd. CLX. (1871), S. 333.

[3] *Ann. d. Chem. u. Pharm.* Bd. LXXXI. (1852), S. 375.

[4] *Zt. f. anal. Chem.* Bd. IV. (1865), S. 264.

(iii) *Seidel's reaction.*[1] A small amount (say ·03 gr.) of the suspected substance is evaporated to dryness in a platinum crucible with a little nitric acid (sp. gr. 1·1—1·2), and the residue is treated with ammonia and a few drops of a solution of strontium acetate. If inosit is present, a greenish colouration is observed, together with a violet precipitate.

THE CANE-SUGAR GROUP.

I. **Saccharose.** (*Cane-sugar.*) $C_{12}H_{22}O_{11}$.

Although it is not found as a constituent of any animal tissue, this sugar possesses no inconsiderable interest in view of the fact that it is a food-stuff which is largely consumed by man, and may constitute in many cases no small part of the total carbohydrates with which the body is supplied.

Cane-sugar is chiefly distinguished from the others by the fact that it does not reduce metallic salts, and does not form a compound with phenyl-hydrazin; but the property which is of greatest interest to the physiologist is the ease with which it may be 'inverted' or converted into equal parts of dextrose and lævulose:—

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 \text{ (dextrose)} + C_6H_{12}O_6 \text{ (lævulose)}.$$

This inversion is readily brought about by treatment with dilute mineral acids at 100°, or even at 40° or below if the action is more prolonged;[2] it is also the result of the action of enzymes, more especially of invertin from yeast, and is characterised experimentally by the change in the rotatory power of the solution, which from being originally dextro-rotatory becomes lævo-rotatory; hence the name 'inversion.' For cane-sugar $(\alpha)_D = +66°$; for lævulose $(\alpha)_D = -100°$. The rotatory power of the latter is largely dependent upon temperature and concentration.

When cane-sugar is injected into the blood-vessels or tissues of an animal it is eliminated in an unaltered condition, and is thus shown to be non-assimilable.[3] On the other hand, it may be introduced in large amounts into the alimentary canal without reappearing externally in the urine. From this it may be concluded that it undergoes some change before or during absorption, and this change is most probably that of inversion. This change may take place in the stomach, partly under the influence of the acid of the gastric juice, but also as the result of the action of a soluble enzyme;[4] it is even more marked in the small intestine, where the active agent is without doubt an enzyme.[1] From this it appears that cane-sugar conforms to the apparently general rule that the carbohydrates leave the alimentary canal as dextrose.

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[1] Dissertation, Dorpat, 1884. Quoted by Fick. (*Pharm. Zt. f. Russl.*) See Abst. in *Ber. d. d. chem. Gesell.* Jahrg. xx. (1887), Ref. Bd. S. 320.

[2] Cf. Wohl, *Ibid.* Jahrg. XXIII. (1890), S. 2087.

[3] Bernard, *Leçons de Physiol. exp.* T. I. 1855, p. 219.

[4] Leube, Virchow's *Arch.* Bd. LXXXVIII. (1882), S. 222. Cf. Hoppe-Seyler,

Cane-sugar readily undergoes a lactic-acid fermentation in presence of sour milk to which zinc oxide is added for the fixation of the acid as it is formed.

2. **Maltose.** $C_{12}H_{22}O_{11} + H_2O$.

This is the sugar which is characteristically formed, together with dextrins, by the action of malt-extract (diastase) on starch-paste. It was first described by Dubrunfaut[2] as arising in this way, but its existence was for some time doubted until firmly established by O'Sullivan.[3] Later researches showed that it is similarly the chief sugar which is formed by the action of saliva and pancreatic juice upon starch-paste or upon glycogen, being accompanied in the case of pancreatic juice by a variable but distinct amount of dextrose if the action of this secretion be prolonged.[4] Maltose is also formed by the action of dilute acids upon starch-paste, but in this case it is difficult to prevent the simultaneous formation of dextrose into which it is readily converted by acids, yielding 98 — 99 p. c. of the latter sugar.[5] It is therefore usually prepared from the products of the action of malt-extract on starch-paste.[6]

Maltose is very soluble in water, also in alcohol, but less so in the latter solvent than is dextrose. It crystallises in fine needles which are however not very easily obtained. Solutions of maltose are dextro-rotatory and reduce metallic salts; it is therefore not easily distinguished from dextrose by merely qualitative tests. As the necessity of discriminating between the two sugars is one of frequent occurrence, the following characteristic differences between their optical and reducing powers are of great importance. For maltose in 10 p. c. solution at 20°C. $(\alpha)_D = +140^\circ$,[7] for dextrose $(\alpha)_D = +52{\cdot}5^\circ$. When maltose is boiled with Fehling's

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Virchow's *Arch.* Bd. x. (1856), S. 144. Koebner, *Diss.* Breslau, (1859). Abst. in Henle u. Meissner's *Jahresb.* 1859, S. 236.

[1] Leube, *Centralb. f. d. med. Wiss.* 1868, S. 289. Paschutin, *Arch. f. Anat. u. Physiol.* Jahrg. (1871), S. 374. Bernard, *Gaz. méd. de Paris*, 1873, p. 200. Brown and Heron, Liebig's *Ann.* Bd. CCIV. (1880), S. 228. *Proc. Roy. Soc.* No. 204, 1880, p. 393. Vella, Moleschott's *Untersuch.* Bd. XIII. (1881), S. 40.

[2] *Ann. Chim. et Phys.* (3) T. XXI. (1847), p. 178.

[3] *Jl. Chem. Soc.* Ser. 2, Vol. x. (1872), p. 579. Cf. Musculus u. Gruber, *Zt. physiol. Chem.* Bd. II. (1878–79), S. 177.

[4] Musculus u. von Mering, *Zt. f. physiol. Chem.* Bd. I. (1877–78), S. 395; II. (1878), S. 403. Külz, Pflüger's *Arch.* Bd. XXIV. (1881), S. 81. Brown and Heron, Liebig's *Ann.* Bd. CXCIX. (1879), S. 165; Bd. CCIV. S. 228. *Proc. Roy. Soc.* No. 204, 1880, p. 393. von Mering, *Zt. f. physiol. Chem.* Bd. V. (1881), S. 185.

[5] Meissl, *Jn. f. pr. Chem.* (2), Bd. XXV. (1882), S. 114.

[6] Soxhlet, *Jn. f. pr. Chem.* (2), Bd. XXI. (1880). Herzfeld, Liebig's *Ann.* Bd. CCXX. (1884), S. 211.

[7] Meissl, *loc. cit.* Brown and Heron make it less $= +135{\cdot}4$.

fluid[1] the amount of cuprous oxide which separates out is only about two-thirds of that which would be reduced by an equal weight of dextrose, or in other words 66 parts of dextrose reduce as much as 100 parts of maltose. Bearing in mind that maltose may be readily converted into dextrose by boiling with dilute acids with a corresponding change of its optical and reducing powers, while dextrose is of course unaltered by this operation, it is easy to base upon the above facts a method of identifying the two sugars. As a further difference between the two it may be stated that Barfoed's reagent[2] is not reduced by maltose, whereas it is by dextrose.[3] In this respect maltose resembles lactose (milk-sugar) which also does not reduce this reagent.

Phenyl-maltosazone. $C_{24}H_{32}N_4O_9$.

This compound of maltose is obtained by the action of phenylhydrazin upon it in presence of acetic acid in the way already described (p. 104) for the preparation of the analogous compound with dextrose. It crystallises readily in minute yellow needles and is characterised by being (unlike phenyl-glucosazone) soluble in about 75 parts of boiling water, and still more soluble in hot alcohol. Its melting point 206° is practically the same as that of phenyl-glucosazone.

The researches of Brown and Heron (see above, p. 59) showed that whereas pancreatic juice rapidly converts starch-paste into maltose and a little dextrose, an extract of the mucous membrane of the small intestine or the tissue itself, while acting but feebly on starch-paste rapidly converts maltose into dextrose. They hence surmised that maltose would be found to be a non-assimilable sugar, requiring like cane-sugar to be converted into the simpler dextrose before absorption. More recent experiments have confirmed this view,[4] for it has been found that if maltose be injected into the blood-vessels it is largely excreted in an unaltered form in the urine.[5] The converting action of extracts of the intestinal mucous membrane is strikingly less than that of the tissue itself; from this it may perhaps be inferred that the change into dextrose takes place rather *during* than previous to absorption. This fact corresponds closely to the well-known views as to the changes which peptones similarly undergo during their passage through the walls of the intestine into the neighbouring blood-vessels (see § 309).

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[1] Solution of hydrated cupric oxide in caustic soda, in presence of the double tartrate of sodium and potassium (Rochelle salt). See Soxhlet, *loc. cit.*

[2] Dissolve 1 part of cupric acetate in 15 parts of water: to 200 c. c. of this solution add 5 c.c. of acetic acid containing 38 p.c. of glacial acid. *Jn. f. pr. Chem.* (2), Bd. vi. (1872), S. 334.

[3] Musculus u. von Mering, *loc. cit.*

[4] But cf. previously Bimmermann, Pflüger's *Arch.* Bd. xx. (1879), S. 201.

[5] Philips, *Diss.* Amsterdam, 1881. See Abst. in Maly's *Bericht.* 1881, p. 60. See also Bourquelot, *Compt. Rend.* T. xcvii. (1883), pp. 1000, 1322; T. xcviii. p. 1604. *Journ. de l'Anat. et de la Physiol.* T. xxii. (1886), p. 161.

3. **Lactose** (Milk-sugar). $C_{12}H_{22}O_{11} + H_2O$.

It is found characteristically and solely in milk, in quantities varying with the class of animal and at different times with the same animal.[1] The percentage is relatively high in human milk. It is also said to occur in the urine of lying-in women and sucklings.[2]

Preparation. The casein is precipitated from diluted milk by the addition of acetic acid. The filtrate from this is boiled to coagulate the remaining proteids, which are then removed by filtration. This final filtrate is then concentrated, and on prolonged standing yields crusts of milk sugar which are purified by recrystallisation from hot water.

It yields, when pure, hard colourless crystals, belonging to the rhombic system (four-sided prisms). It is less soluble in water than dextrose, requiring for solution six times its weight of cold, but only two parts of boiling, water; it is entirely insoluble in alcohol and in ether. It is fully precipitated from its solutions by the addition of basic lead acetate and ammonia.

Solutions of many metallic salts are readily reduced by boiling with lactose, but the reducing power is less than that of dextrose. Thus 1 c. c. of Fehling's fluid which is reduced by 5 mgr. of dextrose requires 6·7 mgr. of lactose provided that certain conditions as to the dilution of the solution, duration of boiling, &c., are attended to.[3] These are important for the accurate volumetric estimation of lactose. The specific rotatory power of lactose is $(\alpha)_D = +52{\cdot}3^\circ$, and is independent of the concentration in solutions which contain up to 35 p. c. at ordinary temperatures. Its rotatory power is thus identical with that of dextrose. It is, however, readily distinguishable from dextrose by its smaller solubility in water, insolubility in alcohol, and incapability of undergoing direct alcoholic fermentation with yeast. It also does not reduce Barfoed's reagent, and in this resembles maltose. When boiled with dilute mineral acids it yields equal molecules of dextrose and galactose (see p. 106), and since the specific rotatory power of the latter of these is high $[(\alpha)_D = +83^\circ]$, this increase of rotatory (and reducing) power on treatment with acids affords a further convenient means of discrimination between lactose and dextrose.

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[1] See Gorup-Besanez, *Lehrb. d. physiol. Chem.* 1878, S. 444. König, *Chem. d. mensch. Nahrungs- u. Genussmittel*, 3 Aufl. (1889), Bd. I. S. 250 *et seq.*

[2] Hofmeister, *Zt. f. physiol. Chem.* Bd. I. (1877), S. 101. See Neubauer u. Vogel, *Analyse d. Harns*, 2 Theil, 1890, S. 48.

[3] Rodewald u. Tollens, *Ber. d. d. chem. Gesell.* 1878, S. 2076. Soxhlet, *Zt. f. prakt. Chem.* (2), Bd. XXI. 1880, S. 227.

Phenyl-lactosazone. $C_{24}H_{32}N_4O_9$.

This compound of lactose with phenyl-hydrazin is formed under conditions similar to those already described for the preparation of the analogous compound of dextrose. It is soluble in 80 — 90 parts of boiling water and melts at about 200°. It crystallises readily in the form of yellow needles which, unlike the crystals of phenyl-maltosazone, are usually aggregated into clusters.

Lactose is readily capable of undergoing a direct lactic fermentation and this occurs characteristically in souring milk. The exciting cause is doubtless ordinarily an organised ferment, but there is also some evidence of the existence in the alimentary canal of an enzyme which can effect the same conversion. The circumstances and products of the conversion are the same as for dextrose and saccharose.

Although *isolated* lactose is unaffected by yeast, milk itself is capable of undergoing, under the influence of certain ferments, an alcoholic fermentation, and this has been employed from very early times by the inhabitants of certain districts of Russia in the preparation of Kumys and Kephir from mare's-milk. Of late years these fluids have attracted much attention in virtue of their supposed therapeutic action in certain wasting diseases. Very little is as yet known as to the real nature of the changes which occur during the fermentation, but they are probably extremely complex and due to the presence of several organised ferments.[1] Kephir ferment is a commercial article in Russia, obtainable at the apothecaries.

The non-assimilability of saccharose and maltose has already been referred to, and experiment has shown that lactose is similarly incapable of assimilation, for when injected into the blood-vessels it appears unaltered in the urine.[2] It is therefore presumably changed in the alimentary canal into some form of sugar which is assimilable, it may be into dextrose and galactose. It does not appear that any such conversion can be markedly observed, if at all, under the action of any of the secretions of the alimentary canal; hence the change may more probably take place, as in the case of maltose, rather during than before the passage of the sugar through the intestinal walls.

This non-assimilability of lactose is certainly remarkable when it is remembered that it is in this form that young animals receive their supply of carbohydrate food. It might more probably have been expected that they should be shielded as far as possible from any avoidable excessive digestive labour by the presentation of a directly assimilable sugar. We cannot as yet offer any other explanation of the observed facts than the one that since lactose is incapable of direct (alcoholic) fermentation, not only is the milk while it is accumulated in the breast less liable to fermentative decomposition, but also the tendency to fermentative disturbance in the alimentary canal of the young animal is largely diminished. Both saccharose (cane-sugar) and maltose[1] are similarly not directly fermentable, and both again in the adult are apparently converted into fermentable dextrose during, or at least, immediately before, absorption. The subject is one which requires further investigation.

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[1] There is an extensive literature on this subject, of which the following are of most comprehensive interest. Biel, *Unters. über den Kumys*, Wien, 1874, and St. Petersburg, 1881. Abst. in Maly's *Bericht.* 1874, p. 166, 1886, p. 159. Struve, *Ber. d. d. chem. Gesell.* Jahrg. 1884, Sn. 314, 1364. Krannhals, *Deutsch. Arch. f. klin. Med.* Bd. xxxv. (1884), S. 18. Hammarsten (Swedish). See Abst. in Maly's *Bericht.* 1886, p. 163.

[2] Dastre, *Compt. Rend.* T. xcvi. (1883), p. 932. *Compt. Rend. Soc. Biol.* (9), T. i. (1889), p. 145. De Jong (Dutch Diss.). See Maly's *Bericht.* 1886, p. 445.

FATTY ACIDS AND FATS, THEIR DERIVATIVES AND ALLIES.

I. Acids of the Acetic Series.

General formula $C_nH_{2n+1}.COOH$ (monobasic).

This, which is one of the most complete homologous series of organic chemistry, runs parallel to the series of monatomic alcohols. Thus formic acid corresponds to methyl alcohol, acetic acid to ethyl (ordinary) alcohol, and so on. The several acids may be regarded as being derived from their respective alcohols by simple oxidation taking place in two stages, the first yielding an aldehyde, the second an acid by direct union of oxygen with the aldehyde.[2] Thus with ethyl alcohol

$$\text{(i)}\ CH_3.CH_2.OH + O = CH_3.COH + H_2O,$$

$$\text{(ii)}\ CH_3.COH + O = CH_3.COOH.$$

The successive members differ in composition by CH_2, and the boiling points rise successively by about 19°C. Similar relations hold good with regard to their melting-points and specific gravities. The acid properties are strongest in those where n has the least value. The lowest members of the series are volatile liquids, acting as powerful acids; these successively become less and less fluid; and the highest members are colourless solids, closely resembling the neutral fats in outward appearance. Consecutive acids of the series present but very small differences of chemical and physical properties, hence the difficulty of separating them: this is further increased in the animal body by the fact that exactly those acids which present the greatest similarities usually occur together.[1]

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[1] Horace Brown. Private communication to author. Cf. v. Mering, *Zt. f. physiol. Chem.* Bd. v. (1881), S 189

[2] The views as to the possible importance of the aldehydes have already been referred to when treating of proteids (see p. 52). It is further interesting to notice that a simple polymerisation, to which it is very prone, of the lowest (meth-) aldehyde H . COH, would yield a substance having the composition of a carbohydrate. This is indeed a view which is held by many as to the mode of formation of starch in plants. Cf. Miller's *Chemistry*, Part III. 1880, Sec. 1, p. 726.

The free acids are found only in small and very variable quantities in various parts of the body; their derivatives on the other hand form most important constituents of the human frame, and will be considered further on.

Some of the lower acids of the series have been obtained by treating proteids with molten caustic potash. They also occur among the products of the putrefaction of proteids, as for instance in old cheese.

Of the primary alcohols from which this series of acids is derived only two have as yet been obtained from animal tissues or secretions, viz. ethyl[2]- and cetyl-alcohol,[3] $C_2H_5 . OH$ and $C_{16}H_{33} . OH$, — the former from muscle, brain, and liver, the latter in union with palmitic acid in spermaceti and the secretion of the caudal glands of birds.

Formic acid. H . COOH.

When pure is a strongly corrosive, fuming fluid, with powerful irritating odour, solidifying at 0° C., boiling at 100° C., and capable of being mixed in all proportions with either water or alcohol. It has been obtained from various parts of the body, such as the spleen, thymus, pancreas, muscles, brain, and blood; in the latter its presence may be due to the action of acids on the hæmoglobin. It also occurs in minute traces in urine. It is excreted by some ants (Formica rufa) in a fairly concentrated form and may be present to the surprisingly large extent of 40 p.c. in the secretion of certain caterpillars.[4] The separation of so acid a fluid from the alkaline cell-substance is remarkable and of considerable interest. When heated with strong sulphuric acid it is decomposed into carbonic oxide and water. It is further characterised by readily effecting the reduction of metallic salts, as of mercury or silver, when heated with their solutions.

Acetic Acid. $CH_3 . COOH$.

It is distinguished by its characteristic odour; its boiling-point is 100° C.; the anhydrous acid solidifies at about 17°. It is soluble in all proportions in alcohol and in water.

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[1] For details on this series see Hoppe-Seyler's *Hdbch. d. phys. path. chem. Anal.* 1883, S. 85 *et seq.*

[2] Rajewski, Pflüger's *Arch.* Bd. XI. (1875), S. 122.

[3] De Jonge, *Zt. f. physiol. Chem.* Bd. III. (1879), S. 225.

[4] Poulton, *The colours of animals,* Internat. Sci. Ser. 1890, p. 274.

It may be formed in the stomach as the result of fermentative changes in the food, and is frequently present in diabetic urine, as also in traces in normal urine. In other organs and fluids it exists only in minute traces.

With ferric chloride it yields a blood-red solution, decolourised by hydrocloric acid. (It differs in this last reaction from sulphocyanide of iron.) Heated with alcohol and sulphuric acid, the characteristic odour of acetic ether (ethyl-acetate) is obtained.

Acetone. $CH_3 . CO . CH_3$.

This substance is the typical member of the general class known as ketones, and may be prepared by the dry distillation of calcium or barium acetate.

Ketones are characterised by containing the group CO (carbonyl) in the same way that the aldehydes are characterised by the group COH, and the acids by the group COOH. The ketones are closely related to the aldehydes and may be regarded as derived from them by displacing the H of the COH group by some monad (alcohol) radicle. They are most usually prepared by the dry distillation of the calcium salts of the appropriate acids. Ketones, like the aldehydes, unite readily and directly with phenyl-hydrazin, yielding a class of compounds, known as hydrazones. (Cf. p. 102.)

Acetone is a volatile liquid, soluble in water, boiling at 56°, and possessed of an agreeable ethereal odour. It may be obtained in considerable quantity by distillation from the urine and blood of diabetic patients and accounts for the peculiar ethereal odour which these frequently evolve.[1] This symptom is of serious prognostic importance, and it has been supposed by many authors that the fatal diabetic coma which rapidly supervenes is caused by the presence of acetone.[2] The urine of diabetic patients frequently exhibits a reddish-violet colouration with ferric chloride, supposedly due to the presence of aceto-acetic acid ($CH_3 . CO . CH_2 . COOH$) which readily yields acetone by its decomposition.

Acetone is also not infrequently found in the urine and breath (?) of children in apparently normal health.[3]

Acetone gives a characteristic reaction with iodine in presence of an alkali (formation of iodoform) and colour-reactions with sodium nitro-prusside and fuchsin.[4]

Propionic acid. $C_2H_5 . COOH$.

This acid closely resembles the preceding one. It possesses a very sour taste and pungent odour; is soluble in water, boils at 141° C., and may be separated from formic and acetic acid by taking advantage of the superior solubility of its lead salt in cold water.

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[1] Von Jaksch, *Ueber Acetonurie u. Diaceturie*, Berlin, 1885. Gives history and literature of the subject. Cf. *Zt. f. physiol. Chem.* Bd. VI. (1882), S. 541.

[2] Cf. Gamgee's *Physiol. Chem.* Vol. I. 1880, p. 168.

[3] Baginsky, *Arch. f. Physiol.* Jahrg. 1887, S. 349.

[4] Consult Neubauer und Vogel, *Harnanalyse*, S. 31.

It occurs in small quantities in sweat, in the contents of the stomach, and in diabetic urine when undergoing fermentation. It is similarly produced, mixed however with other products, during alcoholic fermentation.

It is stated to have been found occasionally in normal urine.

Butyric acid. $C_3H_7 . COOH$.

There are two possible isomeric acids of the general formula $C_3H_7 . COOH$, the *normal* or *primary*, $CH_3 . CH_2 . CH_2 . COOH$ and *iso-* or *secondary*, $CH(CH_3)_2 . COOH$.

Normal butyric acid. An oily colourless liquid, with an odour of rancid butter, soluble in water, alcohol, and ether, boiling at 162° C.

Found in sweat, the contents of the large intestine, fæces, and in urine. It occurs in traces in many other fluids, and is plentifully obtained when diabetic urine is mixed with powdered chalk and kept at a temperature of 35° C. It exists, in union with glycerin as a neutral fat, in small quantities in milk, and gives the characteristic odour to butter which has become rancid.

It is the principal product of the second stage of lactic fermentation (see p. 105), and is ordinarily prepared from this source.

Isobutyric acid. Occurs in fæces and among the putrefactive products from proteids, also in certain fruits such as the banana.

Valeric or Valerianic acid. $C_4H_9 . COOH$.

Four isomeric forms of this acid exist. Of these the one here described is the *isoprimary* $CH(CH_3)_2CH_2 . COOH$. (Isopropylacetic acid.)

An oily liquid, of burning taste and penetrating odour as of decaying cheese; soluble in 30 parts of water at 12°C., readily soluble in alcohol and in ether. Boils at 175° C.

It is found in the solid excrements, and is formed readily by the decomposition, through putrefaction, of impure leucin, ammonia being at the same time evolved; hence its occurrence in urine when that fluid contains leucin, as in cases of acute atrophy of the liver.

Caproic acid. $C_5H_{11} . COOH$.

Caprylic acid. $C_7H_{15} . COOH$.

Capric (Rutic) **acid.** $C_9H_{19} . COOH$.

These three occur together (as fats) in butter, and are contained in varying proportions in the fæces from a meat diet and the first two in sweat. The first is an oily fluid, slightly soluble in water, the others are solids and scarcely soluble in water; they are soluble in all proportions in alcohol and in ether. They may be prepared from butter, and separated by the varying solubilities of their barium salts.

Lauric or Laurostearic acid. $C_{11}H_{23} . COOH$.
Myristic acid. $C_{13}H_{27} . COOH$.

These occur as neutral fats in spermaceti, in butter and other fats. They present no points of interest.

Palmitic acid. $C_{15}H_{31} . COOH$.
Stearic acid. $C_{17}H_{35} . COOH$.

These are solid, colourless when pure, tasteless, odourless, crystalline bodies, the former melting at 62° C., the latter at 69·2° C. In water they are quite insoluble; palmitic acid is more readily soluble in cold alcohol than stearic: both are readily dissolved by hot alcohol, ether, or chloroform. Glacial acetic acid dissolves them in large quantity, the solution being assisted by warming. They readily form soaps with the alkalis, also with many other metals. The varying solubilities of their barium salts afford the means of separating them when mixed:[1] this method may also be applied to many others of the higher members of this series.

These acids in combination with glycerin (see below), together with the analogous compound of oleic acid, form the principal constituents of human fat. As salts of calcium they occur in the fæces and in 'adipocire,' and probably in chyle, blood, and serous fluids, as salts of sodium. They are found in the free state in decomposing pus, and in the caseous deposits of tuberculosis.

The existence of margaric acid, as obtained from natural fats, intermediate to the above two, is not now admitted, since Heintz has shown[2] that it is really a mixture of palmitic and stearic acids. Margaric acid possesses the anomalous melting-point of 59·9° C. A mixture of 60 parts stearic acid and 40 of palmitic acid, melts at 60·3°. A true margaric acid may however be prepared by replacing the group OH in cetyl-alcohol ($C_{16}H_{33} . OH$) by the group COOH.

Adipocire. When animal (proteid) tissues are buried for some time in damp ground or otherwise exposed to moisture in the absence of any free supply of oxygen they are frequently found to have undergone a peculiar change by which they are converted into a waxy or fatty substance. This is known as adipocire. It consists, not of true neutral fats, but of the ammonium, and in some cases calcium, salts of the highest fatty acids palmitic and stearic, or of the free acids themselves.[1] Practically nothing is definitely known as to the agencies and mode of this conversion. It may be the result of a purely chemical change, or perhaps it is more probably due to the action of some micro-organism.[2] On either view of its formation the occurrence of adipocire is of extreme interest as showing a possible direct formation of the higher fatty acids and hence of fats from proteids. It is however supposed by some authors that the adipocire is formed entirely by change and aggregation from the fats present in the tissues at death.[3] This view is probably incorrect.

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[1] Heintz, Poggendorff's *Annal. d. Phys. u. Chem.* Bd. XCII. S. 588.
[2] *Op. cit.*

II. ACIDS OF THE OLEIC (ACRYLIC) SERIES. $C_nH_{2n-1}.COOH$ (monobasic).

The acids of this series bear the same relationship to the olefines (C_2H_4) that those of the acetic do to the paraffins (CH_4). Some of the higher members of the series are found as glycerin compounds in various fats.

They bear an interesting relation to the acids of the acetic series, breaking up when heated with caustic potash into acetic acid and some other member of the same series: — thus,

$$\underset{\text{Oleic acid.}}{C_{17}H_{33}.COOH} + 2KHO = \underset{\text{Potassium acetate.}}{KC_2H_3O_2} + \underset{\text{Potassium palmitate.}}{KC_{16}H_{31}O_2} + H_2.$$

Oleic acid. $C_{17}H_{33}.COOH$.

This is the only acid of the series which is physiologically important. It is found united with glycerin in all the fats of the human body.

When pure it is, at ordinary temperatures, a colourless, odourless, tasteless, oily liquid, solidifying at 4° C. to a crystalline mass. Insoluble in water, it is soluble in alcohol and in ether. It cannot be distilled without decomposition. It readily forms with potassium and sodium hydroxide soaps which are soluble in water: its compounds with most other bases are insoluble. It may be distinguished from the acids of the acetic series by its reaction with nitrous acid which converts it into a solid (elaïdic acid) and by the changes it undergoes when exposed to the air. It may be converted into stearic acid

$$C_{17}H_{33}.COOH + H_2 = C_{17}H_{35}.COOH.$$

THE NEUTRAL FATS.

These may be considered as ethereal salts formed by replacing the exchangeable atoms of hydrogen in the triatomic alcohol glycerin (see below), by the acid radicles of the acetic and oleic series. Since there are three such exchangeable atoms of hydrogen in glycerin, it is possible to form three classes of these ethereal salts; only those, however, which belong to the third class occur as natural constituents of the human body: those of the first and second are of theoretical importance only.

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[1] Ebert, *Ber. d. d. chem. Gesell.* Bd. VIII. (1875), S. 775.

[2] Kratter, *Zt. f. Biol.* Bd. XVI. (1880), S. 455. Lehmann, *Sitzb. d. phys.-med. Gesell.* Würzburg, 1888, S. 19.

[3] Zillner, *Viertelj. f. ger. Med. u. öff. Sanitätsw.* (N.F.) Bd. XLIV. (1885), S. 1.

The following reaction which represents the formation of tri-palmitin from glycerin and palmitic acid is typical for all the others.

$$\underset{\text{Glycerin.}}{C_3H_5\,(OH)_3} + \underset{\text{Palmitic acid.}}{3\,(C_{15}H_{31}\,.\,CO\,.\,OH)} = \underset{\text{Tri-palmitin.}}{C_3H_5\,(C_{15}H_{31}\,.\,CO\,.\,O)_3} + 3\,H_2O.$$

They possess certain general characteristics. Insoluble in water and but slightly in alcohol, they are readily soluble in ether, chloroform, benzol, &c.; they also dissolve one another. They are neutral bodies, colourless and tasteless when pure; they are not capable of being distilled without undergoing decomposition, and yield as a result of this decomposition solid and liquid hydrocarbons, water, fatty acids, and a peculiar substance, acrolein, resulting from the decomposition of the glycerin. (See below.)

They possess no action on polarised light.

They may readily be decomposed into glycerin and their respective fatty acids by the action of caustic alkalis, or of superheated steam.

Palmitin (Tri-palmitin). $C_3H_5\,(C_{15}H_{31}\,.\,CO\,.\,O)_3$.

Palmitin is but slightly soluble in alcohol either cold or hot, readily so in ether, from which, when pure, it crystallises in fine needles; if mixed with stearin it generally forms shapeless lumps, although the mixture may at times assume a crystalline form, and was then regarded as a distinct body, namely margarin. When pure it melts at 62° and solidifies again at 45°.

It is most conveniently obtained from palm-oil by removing the free palmitic and oleic acids by alcohol and repeatedly crystallising the residue from ether.

Stearin (Tri-stearin). $C_3H_5\,(C_{17}H_{35}\,.\,CO\,.\,O)_3$.

This is the hardest and least fusible of the ordinary fats of the body; is also the least soluble, and hence is the first to crystallise out from solutions of the mixed fats. Readily soluble in ether and in *boiling* alcohol. It crystallises usually in square tables or glittering plates. It presents peculiarities in its fusing-points, melting first at 55°, then solidifying as the temperature is further raised, and melting finally and permanently at 71°.

Preparation. From mutton suet, its separation from palmitin and olein being effected by repeated crystallisation from ether, stearin being the least soluble. It is, however, very difficult to obtain it pure by this process.

Olein (Tri-olein). $C_3H_5(C_{17}H_{33}.CO.O)_3$.

Is obtained with difficulty in the pure state, and is then fluid at ordinary temperatures. It is somewhat soluble in alcohol, very soluble in ether. It readily undergoes oxidation when exposed to the air, and is converted by mere traces of nitrous acid into a solid isomeric fat, tri-elaïdin. Olein is saponified with much greater difficulty than are palmitin and stearin.

Preparation. From olive oil, either by cooling to 0° C. and pressing out the olein that remains fluid, or by dissolving in hot alcohol and cooling, when the olein remains in solution while the other fats crystallise out.

The fats which occur in the animal body are mixtures of the above three substances in varying proportions. The normal fat of each animal or class of animals is however characterised by the constant preponderance of one of the three; thus in the fat of man and carnivora palmitin is in excess over the other two. In the fat of herbivora stearin predominates, and in that of fishes olein. Butter contains, in addition to the above, several fats formed by the union of glycerin with the radicles of the lower acids of the acetic series.

There is no doubt that a large part of the fat laid on in the animal body during fattening cannot be accounted for by the fat given in the food, and must hence arise from a conversion of proteids or carbohydrates into fat. (See §§ 506, 507.) The question as to *how* the storage arises from these food-stuffs is one which has given rise to a prolonged controversy. On the one hand Voit and his followers urged that although carbohydrates do lead to a rapid storing of fat in the body, they do so not directly by being themselves converted into fat, but indirectly by protecting the proteids from the metabolism they would otherwise have undergone. According to this view fat is formed from proteids only. Lawes and Gilbert on the other hand took the view that carbohydrates are directly converted into fat. While there is no doubt that proteids can give rise directly to fat as shown by the storage of fat during "nitrogenous equilibrium" (see § 522), there is also now equally no doubt that carbohydrates can lead to a *direct* storage of fat by being themselves converted into fat. This is the incontrovertible outcome of the most recent experiments, which have proved that with a diet rich in carbohydrates, so that the storage of fat is sufficiently rapid, more fat is laid on than could possibly have been formed from the proteids in the food given.[1]

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[1] Meissl u. Strohmer, *Sitzb. d. Wien. Akad.* Bd. LXXXVIII. 1883, III. Abth. July. Tscherwinsky, *Landwirth. Versuchsstat.* Bd. XXIX. (1883), S. 317. Chaniewski, *Zt. f. Biol.* Bd. XX. (1884), S. 179. Rubner, *Ibid.* Bd. XXII. (1886), S. 272. Munk, Virchow's *Arch.* Bd. CI. (1885), S. 91. *Biol. Centralb.* Bd. V. (1885–86), S. 316. See also Voit, *Ibid.* Bd. VI. (1886–87), S. 243.

Glycerin (Glycerol). $C_3H_5(OH)_3$.

As already stated, glycerin is a triatomic alcohol, the neutral fats being ethereal salts formed from it with the radicles of the higher fatty acids and oleic acid.

When pure, glycerin is a viscid, colourless liquid, of a well-known sweet taste. It is soluble in water and in alcohol in all proportions, insoluble in ether. Exposed to very low temperatures it becomes almost solid; it boils at 290° and may be distilled without decomposition in the absence of air.

It dissolves the alkalis and alkaline earths, also many oxides, such as those of lead and copper; many of the fatty acids are also soluble in glycerin.

It possesses no rotatory power on polarised light.

It is easily recognised by its ready solubility in both water and alcohol, its insolubility in ether, its sweet taste, and its reaction with bases. When sufficiently heated, especially in presence of a dehydrating agent, glycerin is decomposed, loses two molecules of water and yields acrolein. $C_3H_5(OH)_3 = C_3H_4O + 2H_2O$. This substance possesses an intensely penetrating, irritating and pungent odour so that its formation enables glycerin to be readily identified. It is the cause of the peculiar smell arising from overheated fats. Chemically it is the aldehyde of allyl alcohol (derived from the olefines) and is intermediate between this substance and acrylic acid, which is a homologue of oleic acid. (See above.)

Glycerin is formed in traces during the alcoholic fermentation of sugar[1]. It is prepared in bulk by distilling in a current of superheated steam the fluid residue left after the saponification of fats with lime.

Soaps.

When neutral fats are heated with lime or caustic alkalis under pressure they are decomposed, the metal combining with the free fatty or oleic acid to form a salt, leaving the glycerin in solution. These salts are called soaps, being soluble in water if the metal is an alkali, insoluble if it is calcium, lead, or other similar metal. The reaction which takes place during the above saponification is as follows.

$$\overset{\text{Tri-sterin.}}{C_5H_5(C_{17}H_{35}\,.\,CO.O)_3} + 3KHO = \overset{\text{Potassium sterate.}}{3(C_{17}H_{35}.COOK)} + \overset{\text{Glycerin.}}{C_3H_5(OH)_3}.$$

A similar decomposition into glycerin and free fatty acid can be effected by pancreatic juice (see p. 64), the acid uniting with the alkali of the juice or of the bile to form a soap. This decomposition is however quantitatively inconsiderable but qualitatively of great importance for the absorption of fats, owing to the extraordinarily great emulsifying power of a mixture of bile, free fatty acids and soluble soaps. The same decomposition takes place when fats, more especially butter, turn rancid.

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[1] Pasteur, *Ann. d. Chem. u. Pharm.* Bd. CVI. (1858), S. 338.

III. Acids of the Glycolic and Oxalic Series.

When one atom of hydrogen in a paraffin is replaced by hydroxyl a primary monatomic alcohol is obtained; if a second atom is replaced a parallel series of diatomic alcohols may be prepared, which are known as glycols. The replacement of a third atom of hydrogen by hydroxyl yields the triatomic alcohols (e. g. glycerin). Further, just as the monatomic alcohols yield acids by oxidation, so also do the glycols; but from the latter two series of acids can be obtained, known respectively as the glycolic and oxalic (succinic) series. Thus at first:

$$\underset{\text{Ethyl-glycol.}}{C_2H_4(OH)_2} + O_2 = \underset{\text{Glycolic acid.}}{CH_2(OH) \, . \, COOH.} + H_2O.$$

By further oxidation a member of the glycolic series can be converted into a member of the oxalic series, thus:

$$\underset{\text{Glycolic acid.}}{CH_2(OH) \, . \, COOH} + O_2 = \underset{\text{Oxalic acid.}}{(COOH)_2} + H_2O.$$

The acids of the glycolic series are monobasic, those of the oxalic dibasic.

The following table exhibits the above relationships in a convenient form.

Paraffin	Alcohol	Acid	Glycol	Acid I	Acid II
Methane	Methyl	Formic			Carbonic [1]
CH_4	$CH_3(OH)$	$H \, . \, COOH$	,,		$CO(OH).(OH)$
Ethane	Ethyl	Acetic	Ethyl-Glycol	Glycolic	Oxalic
C_2H_6	$C_2H_5(OH)$	$CH_3 \, . \, COOH$	$C_2H_4(OH)_2$	$CH_2(OH) \, . \, COOH$	$(COOH)_2$
Propane	Propyl	Propionic	Propyl-glycol	Lactic	Malonic
C_3H_8	$C_3H_7(OH)$	$C_2H_5 \, . \, COOH$	$C_3H_6(OH)_2$	$C_2H_4(OH) \, . \, COOH$	$CH_2(COOH)_2$
Butane	Butyl	Butyric	Butyl-glycol	Oxybutyric	Succinic
C_4H_{10}	$C_4H_9(OH)$	$C_3H_7 \, . \, COOH$	$C_4H_8(OH)_2$	$C_3H_6(OH) \, . \, COOH$	$C_2H_4(COOH)_2$

Glycolic Acid Series.

Lactic (hydroxy-propionic) acid. $C_3H_6O_3$.

This, after carbonic acid, is to the physiologist the most important acid of the series.

If lactic acid is regarded as derived from propionic acid $CH_3 \, . \, CH_2 \, . \, COOH$, it may be noticed at once that two isomeric lactic acids must be capable of being formed from it. These acids will have the following formulæ respectively: $CH_3 . CH(OH) . COOH$ and $CH_2(OH) . CH_2 . COOH$. Of these the first is known as ethylidene-lactic acid, the second as hydracrylic acid.

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[1] This acid is frequently classed in the preceding group of acids as the first of the glycolic series.

In addition to the above a third acid, isomeric with ethylidene-lactic acid is known, namely sarcolactic or paralactic acid. Of these three acids only two occur in the body, hydracrylic being absent. A fourth acid, to which the name of ethylene-lactic acid has been given, has also been described as isomeric with hydracrylic acid. It is however probable that this acid is really acetyl-lactic acid, hydracrylic acid being the true ethylene-lactic acid. (See below.)

The several forms of lactic acid are all syrupy colourless fluids, soluble in all proportions in water and in alcohol, and to a slight extent in ether. They possess an intensely sour taste, and a strong acid reaction. When heated in solution they may partially distil over in the escaping vapour, but are usually decomposed during the process. They form salts with metals, of which those with the alkalis are very soluble and crystallise with difficulty. The calcium and zinc salts are of the greatest importance, as will be seen later on, inasmuch as by their varying solubilities they afford a means of separating the several acids each from the other.

1. **Ethylidene-lactic acid.** $CH_3 . CH(OH) . COOH$.

This is the ordinary form of the acid, obtained characteristically as the chief product of the lactic fermentation of sugars (see p. 105).

From this source it may be readily prepared by adding a little old cheese and sour milk to a solution of cane sugar to which some carbonate of zinc is added. The whole is kept warmed to 40° or 45° for ten days or a fortnight, being vigorously stirred at frequent intervals. The lactic acid is fixed as a lactate by the zinc salt as fast as it is formed, this removal of free acid being essential to the progress of the fermentation which does not take place in an acid solution. The crusts of zinc-lactate formed during the above process are purified by recrystallising, the acid is then liberated from the compound by the action of sulphuretted hydrogen, and extracted by shaking up with ether, in which it is soluble. By a similar process lactic acid may be readily obtained from lactose.

Lactic acid occurs in the contents of the stomach and intestine, more particularly during a diet rich in carbohydrates, and may be readily formed by the digestion of gastric mucous membrane with solutions of dextrose or saccharose.[1] According to Heintz[2] it is found also in muscles, and according to Gscheidlen[3] in the ganglionic cells of the grey substance of the brain.

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[1] Maly, *Ann. d. Chem. u. Pharm.* Bd. CLXXIII. (1874), S. 227.

[2] *Ann. d. Chem. u. Pharm.* Bd. CLVII. (1871), S. 314.

[3] Pflüger's *Archiv*, Bd. VIII. (1873–74), S. 171.

The most important salts of this acid are those of zinc and calcium.

Zinc lactate. Zn $(C_3H_5O_3)_2 + 3H_2O$. Soluble in 53 parts of water at 15° ; in 6 parts at 100°. Almost insoluble in alcohol.

Calcium lactate. CA $(C_3H_5O_3)_2 + 5H_2O$. Soluble in 9·5 parts of cold water; soluble in all proportions in boiling water. Insoluble in cold alcohol.

2. **Sarcolactic acid.**

This form of the acid is isomeric with the preceding one. In its general chemical behaviour as tested by the various decompositions it can undergo it is found to be identical with ethylidene-lactic acid, the sole observable difference being in the different solubility of its calcium and zinc salts. But both sarcolactic acid and its salts differ strikingly from the preceding acid and its salts as regards their physical properties, for the former exert a distinct rotatory action on polarised light while the latter do not. This peculiar kind of isomerism, chemical identity with physical difference, has been called 'physical isomerism' to distinguish it from the ordinary form of chemical isomerism. It is now more usually and correctly called 'stereochemical isomerism' in accordance with the theory which is held as to the nature and cause of the phenomenon. (See below.)

This acid has not yet been prepared synthetically and is only known as occurring characteristically in muscles[1] to which it gives their acid reaction,[2] and in blood.[3] In the latter it is found more particularly, as might be expected, after the muscles have been in a state of contracting activity.[4] It is also found in urine, very markedly in cases of phosphorus poisoning, and in the same excretion after violent muscular exertion,[5] or artificial stimulation of groups of muscles,[6] and very strikingly after extirpation of the liver in birds,[7] and frogs.[8] It is also stated to be formed in variable and slight amount during the lactic fermentation of dextrose.[9] Lactic acid has also been frequently described as a constituent of various pathological fluids; in these cases it is probable that the acid is often the sarcolactic acid.[10]

As occurring characteristically in muscles it is hence found in large quantities in Liebig's 'extract of meat' which is the most convenient source for its preparation.[1]

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[1] Wislicenus, *Ann. d. Chem. u. Pharm.* Bd. CLXVII. (1873), S. 302.

[2] Liebig, *Ann. d. Chem. u. Pharm.* Bd. LXII. (1847), S. 326.

[3] Gaglio, *Arch. f. Physiol.* Jahrg. 1886, S. 400.

[4] Spiro, *Zt. f. physiol. Chem.* Bd. I. (1877), S. 111. Cf. Von Frey, *Arch. f. Physiol.* Jahrg. 1885, S. 557. Also Marcuse, *loc. cit.* below.

[5] Colasanti and Moscatelli. See ref. in Maly's *Bericht.* 1887, S. 212.

[6] Marcuse, Pflüger's *Arch.* Bd. XXXIX. (1886), S. 425.

[7] Minkowski, *Centralb. f. d. med. Wiss.* 1885, No. 2. *Arch. f. exp. Path. u. Pharmakol.* Bd. XXI. (1886), S. 40.

[8] Marcuse, *loc. cit.* But see Nebelthau, *Zt. f. Biol.* Bd. XXV. (1889), S. 123.

[9] Maly, *Ber. d. d. chem. Gesell.* Jahrg. 1874, S. 1567.

[10] Cf. Maly. Abst. in Maly's *Jahresb.* 1871, S. 333. Fluid from ovarial cyst.

Liebig's extract is dissolved in four parts of warm water. To this solution two volumes of 90 p. c. alcohol are added and the precipitate is removed by filtration. The filtrate, after concentration, is again precipitated with four volumes of alcohol. The filtrate from this second precipitate is finally concentrated, acidulated with sulphuric acid, and extracted with excess of ether which dissolves out the sarcolactic acid. On evaporating off the ether and dissolving the residue in water, the pure acid may be obtained by forming its zinc salt, which is purified by crystallisation and decomposed by sulphuretted hydrogen. For the method of detecting and separating this acid from urine see Salkowski and Leube.[2]

The zinc and calcium salts of sarcolactic acid are much more soluble both in water and alcohol than are those of ethylidene-lactic acid.

Zinc sarcolactate. Zn $(C_3H_5O_3)_2 + 2H_2O$. Soluble in 17·5 parts of water at 15° or 964 parts of boiling 98 p. c. alcohol.

Calcium sarcolactate. Ca $(C_3H_5O_3)_2 + 4H_2O$ [? $4\frac{1}{2}$ H_2O]. Soluble in 12·4 parts of cold water, soluble in all proportions in boiling water or alcohol.

The *free* acid is dextro-rotatory, but the true value of $(\alpha)_D$ is unknown owing to uncertainty as to the purity of the acid. The salts on the other hand are all lævo-rotatory. For the zinc salt, when one part is dissolved in 18 of water $(\alpha)_D = -7{\cdot}6°$.

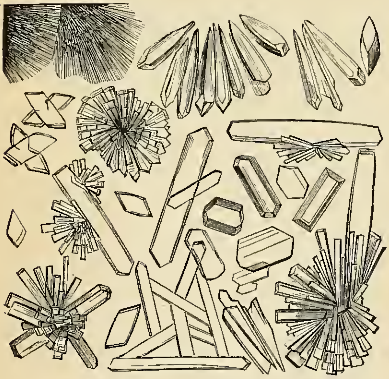

FIG. 2. ZINC SARCOLACTATE. (After Kühne.)

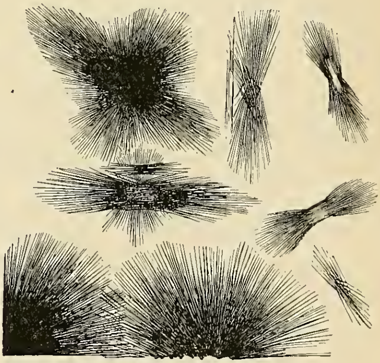

FIG 3 CALCIUM SARCOLACTATE. (After Kuhne.)

Both this acid and the preceding one yield an intense yellow colouration when added to an extremely dilute (almost colourless) solution of ferric chloride. This reaction is sometimes useful.[3]

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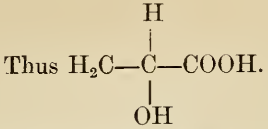
[1] See Gamgee, *Physiol. Chemistry*, Vol. I. 1880, p. 361.
[2] *Die Lehre vom Harn*, 1882, S. 125.
[3] Uffelmann, *Arch. f. klin. Med.* Bd. XXVI. (1880), S. 431.

When the formula of ethylidene-lactic acid is examined it is found to contain what is known as an asymmetric carbon atom: that is to say, an atom of carbon whose affinities are saturated by four dissimilar radicles. Thus



$$\begin{array}{c}H\\ |\\ H_2C—C—COOH.\\ |\\ OH\end{array}$$

According to the hypothesis of Van't Hoff and Le Bel such a substance must be possessed of optically active properties, since all substances which do rotate the plane of polarised light contain an asymmetric carbon atom. It is known however in certain cases, as for instance racemic acid, that although the substance contains one (or more) asymmetric carbon atoms it may still be optically inactive since it is composed of a mixture of isomeric bodies possessing equal and opposite rotatory powers. From this point of view it is probable that ethylidene-lactic acid may be such a mixture, and that at present only one of the optically active isomers of which it is composed has been obtained, viz. sarcolactic acid.

In support of this view it is interesting to notice that a dextrorotatory lactic acid can be obtained from the optically inactive ethylidene-lactic acid, by applying to its ammonium salt Pasteur's method for the separation of a mixture of isomeric substances whose rotatory powers are equal and opposite. This consists in growing the organism Penicillium glaucum in a dilute solution of the mixture; one of the isomers is found to be more readily destroyed by the plant than is the other, so that at a certain stage only one is left in solution.[1] When treated in this way ethylidene-lactic acid yields a dextrorotatory solution.[2] When a current of dry air is passed through sarcolactic (or ethylidene-lactic) acid heated to 150°, two molecules of the acid lose two molecules of water and yield a solid crystalline substance known as lactide $(C_3H_4O_2)_2$. When boiled with water this is reconverted into optically inactive lactic acid, thus effecting the reconversion of the optically active into the inactive form of the acid.

The Van't Hoff-Le Bel hypothesis of what was originally called 'physical' isomerism is based upon considerations of the *spacial* relationships of the constituents of an organic substance; hence the more recent use of the expression 'stereochemical' instead of 'physical.'[3]

The acid reaction of dead muscle is undoubtedly due to the presence of sarcolactic acid, as was first clearly shown by Liebig in 1847.[4] In certain cases the reaction of muscle which is still irritable may become acid, and this has usually been regarded as due to the development of this acid during its activity. In recent times, notwithstanding the evidence of the production of large amounts of sarcolactic acid during muscular contraction (see above), the view has been put forward that the acid reaction of contraction is due rather to other substances, as for instance acid phosphates, than to the acid.[1] This view is by no means proved and is incompatible with the preponderating evidence of the researches already quoted on the relationships of this acid to muscular activity, and of more recent observations.[2] It is possible that the acid reaction of active muscle is of complex origin, being partly due to lactic acid, which by acting on an alkaline phosphate may convert it into an acid salt, while finally there is an excess of the lactic acid, most marked in rigor.

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[1] *Compt. Rend.* T. LI. (1860), p. 153.

[2] Lewkowitsch, *Ber. d. d. chem. Gesell.* Jahrg. 1883, S. 2720.

[3] See Miller's *Elements of Chem.* (Armstrong and Groves), Part III. Sec. 1 (1880), p. 983, for details of the Van't Hoff-Le Bel hypothesis.

[4] That living (irritable) muscle in a state of rest is really alkaline was first demonstrated by Du Bois Reymond in 1859. *Monatsber. d. Berl. Akad.* 1859, S. 288. See his *Gesammel. Abhdl.* Bd. II. 1877, S. 3.

There is but little doubt that the glycogen normally present in muscles is diminished in amount during their contracting activity. and it has been frequently urged that the acid reaction of muscle is due to the formation of sarcolactic acid from this glycogen. This view seems to rest entirely on the fact that during activity glycogen disappears and lactic acid is formed, but is devoid of convincing experimental evidence. It is known that a muscle free from all glycogen can become acid during activity, and bearing in mind that the acidity of active muscle is proportional to its power of doing work, and to the work it is called upon to do,[3] it is most probable that the lactic acid is a product of the breaking down of the complex (nitrogenous) molecule whose decomposition is the source of the energy which the muscle can set free.[4] Glycogen is according to this view to be regarded rather as a convenient accessory to the activity than as either the basis of this activity or of the lactic acid which arises during the activity.

3. **Ethylene-lactic acid.** $CH_2(OH) . CH_2 . COOH$.

This acid has been usually described as accompanying sarcolactic acid in extracts of muscles, and as being isolable from this by taking advantage of the varying solubilities of the zinc salts of the two acids.[5]

More recent researches have however made it probable that what has usually been described as ethylene-lactic acid, obtainable from muscle-extract, is really acetyl-lactic acid, $CH_3 . CH (C_2H_3O_2) COOH$, the true ethylene-lactic acid being hydracrylic acid, which does not occur in the animal body.[6]

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[1] Astaschewsky, *Zt. f. physiol. Chem.* Bd. iv. (1880), S. 397. Weyl u. Zeitler, *Ibid.* Bd. vi. (1882), S. 557.

[2] Werther, Pflüger's *Arch.* Bd. xlvi. (1890), S. 63. Cf. Warren, Pflüger's *Arch.* Bd. xxiv. (1881), S. 391.

[3] Heidenhain, *Mechanische Leistung Wärmeentwick. u. Stoffumsatz bei der Muskelthätigkeit.* Leipzig, 1864. Ranke, *Tetanus.* Leipzig, 1865. Hermann, *Unters. ü. d. Stoffwechsel d. Muskeln.* Berlin, 1867.

[4] Cf. Werther, *loc. cit.* S. 85. Halliburton, *Jl. Physiol.* Vol. viii. (1887), p. 154.

[5] Wislicenus, *Ann. d. Chem. u. Pharm.* Bd. clxvii. (1873), S. 302.

[6] Siegfried, *Ber. d. d. chem. Gesell.* Jahrg. 1889, S. 2711.

Hydroxy-butyric acid.[1] $CH_3 . CH(OH) . CH_2 . COOH$.

This acid is the next homologue to the lactic acids in the glycolic series. It is frequently found in the urine of acute diabetes, usually accompanied by aceto-acetic acid [$CH_3 . CO . CH_2 . COOH$]. The pure acid is sirupy and lævo-rotatory. $(\alpha)_D = -23{\cdot}4$. For its separation from urine and estimation see Külz[2] and Stadelmann.[3]

OXALIC ACID SERIES.

Oxalic acid. $(CO . OH)_2$.

This acid does not occur in the free state in the human body. Calcium oxalate, however, is a not unfrequent constituent of urine, and enters into the composition of many urinary calculi, the so-called mulberry calculus consisting almost entirely of it, and it is very commonly found in urinary deposits. As ordinarily precipitated from solutions of calcium salts by the addition of a salt of oxalic acid, the calcium oxalate is usually amorphous. To obtain it in the crystalline form dilute solutions of the two reagents must be allowed to mix very slowly, as by diffusion. In urine the case is different; the oxalate is at first in dilute solution, probably dissolved by the sodium dihydric phosphate (NaH_2PO_4) to which the acidity is normally due. On standing the urine cools and the oxalate separates out in a crystalline form, viz. rectangular octohedra, which is characteristic and striking, and usually unlike that of any other constituent of urinary deposits.

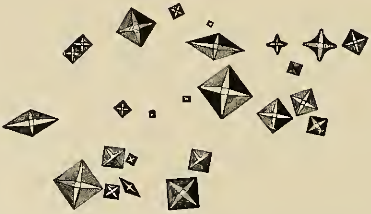

FIG. 4. CALCIUM OXALATE. (After Funke.)

In some cases it presents the anomalous forms of rounded lumps, dumb-bells, or square columns with pyramidal ends, but these forms are uncommon.

The crystals are insoluble in ammonia and acetic acid, but readily soluble in hydrochloric or other mineral acid, also slightly so in solutions of acid phosphates and urates of sodium. The above characteristics serve to identify this salt, but in practice the microscopical appearance is usually of most use.

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[1] See Neubauer u. Vogel, *Analyse d. Harns*, 1890, S. 110.

[2] *Zt. f. Biol.* Bd. XXIII. (1887), S. 329.

[3] *Ibid.* S. 456.

Succinic acid. $COOH.CH_2.CH_2.COOH$.

This is the third acid of the oxalic series, being separated from oxalic acid by the intermediate malonic acid, $CH_2(COOH)_2$. It may occur in the spleen, the thymus, and thyroid bodies, hydrocephalic and hydrocele fluids. It has also been stated to occur normally in urine, but this is very doubtful,[1] as also is the statement that it is found in this excretion after taking food rich in asparagin, e. g. asparagus.[2] It is obtained as a product of the putrefaction of proteids.[3]

Succinic acid crystallises most usually in the form of large four-sided prisms, occasionally as rhombic tables. It is soluble in about 20 parts of cold water, much more so in hot; it is also soluble in alcohol, more especially if hot, and is but very slightly so in ether.

The crystals melt at 180° C., and boil at 235° C., being at the same time decomposed into the anhydride and water. The alkali salts of this acid are soluble in water, insoluble in alcohol and in ether.

Preparation. Apart from the synthetic methods, it may readily be obtained by the fermentation of malic[4] or tartaric[5] acids, which are closely related to succinic, the former being hydroxy-succinic, $COOH.CH_2.CH(OH).COOH$, and the latter dihydroxy-succinic acid, $COOH.CH(OH).CH(OH).COOH$.

Some of the amido-derivatives of succinic acid, viz. asparagin and aspartic acid, are of considerable interest; they will be described later on.

Cholesterin. $C_{26}H_{44}O$ or $C_{25}H_{42}O$.[6]

This substance is described here rather for the sake of convenience than from its possessing any relationship to those which have preceded it.

Cholesterin is the only alcohol which occurs in the human body in the free state. (The triatomic alcohol glycerin is always found combined as in the fats; and cetyl-alcohol is obtained only from spermaceti.) It is a glittering white crystalline substance, soapy to the touch, crystallising in fine needles from its solution in ether, chloroform, or benzol; from its hot alcoholic solutions it is deposited on cooling in rhombic tables; this is the characteristic form and of great importance for the identification of cholesterin.

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[1] Salkowski, Pflüger's *Arch.* Bd. IV. (1871), S. 94.

[2] v. Longo, *Zt. f. physiol. Chem.* Bd. I. (1877), S. 213.

[3] Salkowski, E. u. H., *Ber. d. d. chem. Gesell.* 1880, S. 189.

[4] Liebig, *Ann. d. Chem. u. Pharm.* Bd. LXX. (1849), Sn. 104, 363.

[5] König, *Ber. d. d. chem. Gesell.* 1882, S. 172.

[6] Hesse, *Ann. d. Chem. u. Pharm.* Bd. CXCII. (1878), S. 175. Schulze u. Barbieri, *Jn. f. prakt. Chem.* Bd. XXV. (1882), Sn. 159, 458.

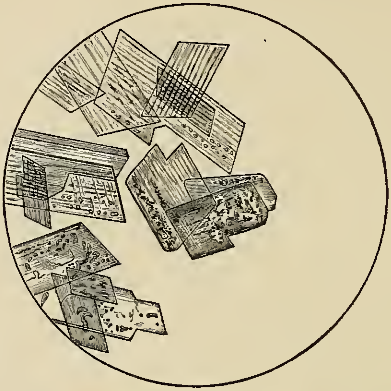

Fig. 5. Cholesterin Crystals. (After Funke.)

When dried it melts at 145°, and distils in closed vessels at 360°. It is quite insoluble in water and in cold alcohol, but soluble in solutions of bile salts.

Solutions of cholesterin possess a left-handed rotatory action on polarised light, $(\alpha)_D = -3{\cdot}5$ in ethereal solution, $= -37°$ in chloroformic.

Cholesterin occurs in small quantities in the blood and many tissues, and is present in abundance in the white matter of the cerebro-spinal axis and in nerves. It is a constant constituent of bile, and forms frequently nearly the whole mass of some gall-stones. It is found in many pathological fluids, hydrocele, the fluid of ovarial cysts, &c., also in fæces and milk.[1] It also occurs in the substance of the crystalline lens, more especially in ‘cataract.’

Preparation. Gall-stones supply the most convenient source of cholesterin. These are pounded, extracted with boiling water and dissolved in boiling alcohol. The solution is filtered through a heated filter, and the cholesterin separates out in a fairly pure condition as the filtrate cools. It is purified by resolution in boiling alcohol to which some caustic soda has been added; from this it again separates on cooling, and is finally washed with cold alcohol and water.

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[1] Tolmatscheff, Hoppe-Seyler's *Med. Chem. Untersuch.* Hf. 2 (1867), S. 272. Schmidt-Mülheim, Pflüger's *Arch.* Bd. xxx. (1883), S. 384.

Cholesterin is characterised, apart from its crystalline form, by some striking reactions which may be obtained even with microscopic quantities.

(i) When the crystals are treated with concentrated sulphuric acid they usually turn violet or red. On the addition of a little iodine the play of colours is very marked, the crystals being variously coloured, — blue, red, green, violet.[1]

(ii) When dissolved in chloroform, the solution turns blood-red on the addition of an equal volume of concentrated sulphuric acid: this turns to blue, green, and finally yellow, the change of colour being very rapid if the solution is freely exposed to the air in an open dish. The sulphuric acid under the chloroform exhibits a green fluorescence.[2]

(iii) When evaporated to dryness on porcelain with a few drops of concentrated nitric acid, a yellow residue is obtained, which turns red if treated, while still hot, with ammonia.

COMPLEX NITROGENOUS FATS AND THEIR DERIVATIVES.[3]

Lecithin. $C_{44}H_{90}NPO_9$.

Occurs widely spread throughout the body. Blood (red-corpuscles),[4] bile, and serous fluids contain it in small quantities, while it is a conspicuous component of the brain, nerves, yolk of egg, semen, pus, white blood-corpuscles, and the electrical organs of the ray. It occurs also in yeast[5] and other vegetable cells, and in small amount in milk.[6]

The presence of lecithin in the red blood-corpuscles may prove to be of no inconsiderable importance in connection with the possible fixation by them of carbonic anhydride.[7] Setschenow has shown that lecithin acts like a base towards carbonic anhydride, each molecule of the substance being able to combine loosely with approximately one molecule of the anhydride (·092 gr. lecithin fixes 2·7 cc. of CO_2) at a partial pressure of 56 mm.[8] Further, it is stated that red blood-corpuscles contain about ·75 p.c. of lecithin,[1] hence 100 grm. red corpuscles might therefore hold in loose combination rather more than 22 cc. of carbonic anhydride. It is of course possible that the lecithin does not exist in a free state in the unaltered corpuscles, and is therefore in living blood incapable of playing the part above ascribed to it. Still the possibility that it may do so is distinctly worth some consideration, bearing in mind how scanty is our knowledge of the real conditions which determine the fixation of carbonic anhydride by the blood.

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[1] See figures in Funke, *Atlas d. physiol. Chem.* Leipzig, 1858, Taf. VI. Fig. 2, 3. This work should be consulted for the crystalline forms of all physiologically important substances. See also Ultzmann u. Hoffmann, *Atlas d. Harnsedimente.* Wien, 1872.

[2] Cf. Burchard, *Inaug. Diss.* Rostock, 1889. Abst. in *Ber. d. d. chem. Gesell.* Ref. Bd. 1890, S. 752.

[3] For a fuller account of the several substances comprised in this group see Gamgee, *Physiol. Chemistry*, Vol. I. (1880), p. 425 et seq.

[4] Cf. Hoppe-Seyler, *Physiol. Chem.* 1877, S. 402.

[5] Hoppe-Seyler, *Zt. f. physiol. Chem.* Bd. II. (1878), S. 427; Bd. III. S. 374.

[6] Tolmatscheff, also Schmidt-Mülheim, *loc. cit.* (sub Cholesterin).

[7] Al. Schmidt, *Ber. d. sächs. Gesell. d. Wiss.* Bd. XIX. (1867), S. 30. Zuntz, *Centralb. f. d. med. Wiss.* 1867, S. 529. Setschenow, *Ibid.* 1877, S. 625; 1879, S. 369; Pflüger's *Arch.* Bd. VIII. 1874, S. 20. Frédéricq, *Compt. Rend.* T. LXXXIV. 1877, p. 661. Mathieu et Urbain, *Ibid.* p. 1305.

[8] Setschenow, *Mém. de l'Acad. Imp.* St. Petersb. T. XXVI. (1879), No. 13, p. 19.

When pure, it is a colourless, slightly crystalline substance, which can be kneaded, but often crumbles during the process. It is readily soluble in cold, exceedingly so in hot alcohol; ether dissolves it freely though in less quantities, as also do chloroform, fats, benzol, carbon, disulphide, &c. It is often obtained from its alcoholic solution, by evaporation, in the form of oily drops. It swells up in water and during the action, as observed under the microscope, extremely curious curling filamentous processes can be seen to protrude from the edge of the solid. These are the so-called ‘myelin forms.’[2]

Preparation. Usually from the yolk of egg, where it occurs in union with vitellin. Its isolation is complicated, and the reader is referred to Hoppe-Seyler.[3]

Lecithin is easily decomposed; not only does this decomposition set in at 70° C., but the solutions, if merely allowed to stand at the ordinary temperature, acquire an acid reaction, the substance being decomposed. Acids and alkalis, of course, effect this much more rapidly. If heated with baryta water it is completely decomposed, the products being cholin, glycerinphosphoric acid, and barium stearate. This may be thus represented:—

$$\underset{\text{Lecithin.}}{C_{44}H_{90}NPO_9} + 3H_2O = \underset{\text{Stearic acid.}}{2C_{18}H_{36}O_2} + \underset{\text{Glycerinphosphoric acid.}}{C_3H_9PO_6} + \underset{\text{Cholin.}}{C_5H_{15}NO_2}.$$

When treated in an ethereal solution with dilute sulphuric acid, it is merely split up into cholin and distearyl-glycerinphosphoric acid. Hence it has frequently been regarded as a sort of salt of cholin with distearyl-glycerinphosphoric acid. It appears however more probable from the most recent researches that it is really an ethereal compound of this acid with the cholin.[4] It appears also that there probably exist other analogous compounds in which the radicles of oleic and palmitic acids take part.

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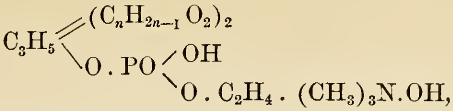
[1] Hohlbeck, Ref. in Hoppe-Seyler, *Physiol. Chem.* 1877, S. 402.

[2] See M‘Kendrick, *General Physiology*, 1888, p. 19.

[3] *Hdbch. d. phys.-path. chem. Anal.*, 1883, S. 166.

[4] Hundeshagen, *Jn. f. prakt. Chem.* Bd. XXVIII. (1883), S. 219. Gilson, *Zt. f. physiol. Chem.* Bd. XII. (1888), S. 585.

In accordance with these views the constitution of lecithin may be most adequately represented by the following formula:—



$$C_3H_5\begin{cases}(C_nH_{2n-1}O_2)_2\\ O.PO\begin{cases}OH\\ O.C_2H_4.(CH_3)_3N.OH,\end{cases}\end{cases}$$

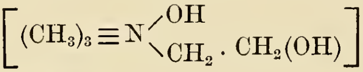
where $C_nH_{2n-1}O_2$ represents the radicle of a fatty acid which in ordinary lecithin appears to be that of stearic, viz. $C_{18}H_{35}O$.

Glycerinphosphoric acid. $C_3H_9PO_6.[C_3H_5.(OH)_2.O.PO(OH)_2]$.

Occurs as a product of the decomposition of lecithin, and hence is frequently found in those tissues and fluids in which the latter is present. It may occur occasionally in urine.[1]

The acid is dibasic and forms salts which are usually, so far as they are known, soluble in cold water, but the lead salt is an exception to this rule and may hence be used as a precipitant. The salts are insoluble in alcohol.

It may be prepared by the decomposition of lecithin when boiled with caustic alkalis or baryta. It may also be synthetised by the direct action of phosphoric anhydride or glacial phosphoric acid on glycerin. The formation by this method may be regarded as resulting from the union of one molecule of glycerin with one of phosphoric acid and elimination of one molecule of water.



Cholin. $C_5H_{15}NO_2.\left[(CH_3)_3\equiv N\begin{cases}OH\\ CH_2.CH_2(OH)\end{cases}\right]$, trimethyloxyethyl-ammonium hydroxide.

Discovered by Strecker[2] among the products of the decomposition of pigs'-bile and subsequently of ox-bile, whence the name cholin. It does not occur in the free state except as a product of the decomposition of lecithin, but has been recently obtained in extracts of the suprarenals.[3] It is a colourless fluid, of oily consistence, possesses a strong alkaline reaction, and forms with acids very deliquescent salts. The salts with hydrochloric acid and with the chlorides of platinum and of gold are the most important.

Cholin is a most unstable body, mere heating of its aqueous solution sufficing to split it up into glycol, trimethylamin and ethylene oxide.

Since it is a product of the decomposition of lecithin it is best prepared from the yolk of egg.[1] The process is elaborate but consists roughly in decomposing the residue of the yolk, left after complete extraction with alcohol and ether, by boiling it for at least an hour with caustic baryta. At the end of this period the barium is precipitated by a stream of carbonic acid, the filtrate is concentrated, extracted with absolute alcohol, and from this solution the cholin is precipitated as a salt by the addition of platinum chloride. It is finally separated from this salt by means of sulphuretted hydrogen.

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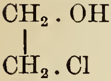
[1] Sotnitschewsky, *Zt. f. physiol. Chem.* Bd. IV. (1880), S. 214. But see also Robin, *Arch. de Pharm.* T. II. p. 532, and *Chem. Centralb.* 1888, S. 186.

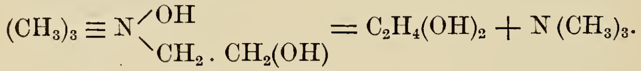
[2] *Ann. d. Chem. u. Pharm.* Bd. CXXIII. (1862), S. 353; Bd. CXLVIII. (1868), S. 76.

[3] Marino-Zuco, *Rend. d. R. accad. d. Lincei*, 1888, p. 835.

Wurtz[2] has obtained it synthetically, first by the action of glycol  $\begin{array}{l} CH_2 . OH \\ | \\ CH_2 . Cl \end{array}$ chlorhydrin on trimethylamine, and then by that of ethylene oxide on a concentrated aqueous solution of trimethylamine.

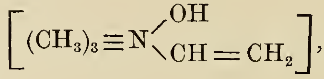
Cholin when pure is an oily liquid with a strong alkaline reaction soluble in alcohol or ether. It yields crystalline compounds with acids and some salts of which the double salts formed with hydrochloric acid and the chlorides of either gold or platinum crystallise readily and are employed for the detection and separation of the base. The platinum salt is readily soluble in water, insoluble in alcohol. The gold salt is but slightly soluble in cold water, but soluble in hot alcohol.

When boiled in concentrated solution cholin is decomposed into glycol and trimethylamine.



$$(CH_3)_3 \equiv N \begin{array}{l} \diagup OH \\ \diagdown CH_2 . CH_2(OH) \end{array} = C_2H_4(OH)_2 + N(CH_3)_3.$$

By oxidation with concentrated nitric acid it yields the extremely poisonous alkaloid muscarin $C_5H_{15}NO_3$.[3] Cholin is itself possessed of poisonous properties, and arising as it does from the decomposition of lecithin and protagon is now recognised as one of the alkaloidal products or ptomaines (see below) which occur in putrefying animal tissues.[4]

Neurin. $C_5H_{13}NO$.  $\left[(CH_3)_3 \equiv N \begin{array}{l} \diagup OH \\ \diagdown CH = CH_2 \end{array} \right]$, trimethylvinyl-ammonium hydroxide.

This substance is closely related to cholin both in composition and origin, but is much more powerfully toxic than that body.

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[1] Diakonow, for ref. and details see Hoppe-Seyler's *Hdbch. d. phys.-path. chem. Anal.* 1883, S. 163.

[2] *Ann. d. Chem. u. Pharm.* Supl.-Bd. VI. Sn. 116, 201. Cf. Baeyer, *Ibid.* Bd. CXL. (1866), S. 306.

[3] Schmiedeberg u. Harnack, *Arch. f. exp. Path. u. Pharm.* Bd. VI. (1876), S. 101. Cf. Berlinerblau, *Ber. d. d. chem. Gesell.* Jahrg. XVII. (1884), S. 1139. But see also Böhm, *Arch. f. exp. Path. u. Pharm.* Bd. XIX. (1885), S. 87.

[4] Brieger, *Zt. f. klin. Med.* Bd. X. (1885), S. 268. See also Brieger's works referred to below, sub Ptomaines.

It was first described as a product of the decomposition of protagon by caustic baryta,[1] and until recently the names cholin and neurin were applied interchangeably to the basic product of the action of baryta on lecithin or protagon first described under the name cholin.[2] The researches of Brieger have however shown that neurin differs distinctly both in composition and properties from the older cholin, and have further identified it as one of the most commonly occurring and actively toxic of the alkaloidal basic products of the putrefactive decomposition of animal tissues known under the name of the ptomaines[3] (see below). Like cholin it is in the pure state a sirupy fluid, with strongly alkaline reaction and is extremely soluble in water. It forms with hydrochloric acid and platinum chloride characteristic double salts which crystallise readily. The double salt which neurin forms with gold chloride crystallises in yellow needles; it is but slightly soluble in cold water, though soluble in hot water

Protagon. $C_{160}H_{308}N_5PO_{35}$ (?).

A crystalline substance, containing nitrogen and phosphorus, obtained by Liebreich[4] from the brain and regarded by him as its principal constituent. The researches of Hoppe-Seyler and Diakonow tended to show that protagon was merely a mixture of lecithin and cerebrin. A repetition of Liebreich's experiments has however led Gamgee and Blankenhorn[5] to confirm the truth of his results, and further confirmation has been afforded still more recently.[6] Protagon appears to separate out from warm alcohol on gradual cooling in the form of very small needles, often arranged in groups: it is slightly soluble in cold, more soluble in hot alcohol, and in ether. It is insoluble in water, but swells up and forms a gelatinous mass. It melts at 200° and forms a brown sirupy fluid.

Preparation. Finely divided brain substance, freed from blood-vessels and connective tissue, is digested at 45° C. with alcohol (85 p.c.) as long as the alcohol extracts anything from it. The united extracts are filtered while hot, and the protagon separates out from the filtrate on cooling to 0°. It is next thoroughly extracted with ether to get rid of all cholesterin and other bodies soluble in ether, and finally purified by repeated crystallisation from warm alcohol.

By treatment with boiling solution of caustic baryta protagon is decomposed, yielding the several products which result from the decomposition of lecithin under the same conditions, together with an additional product known as cerebrin.

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[1] Liebreich, *Ber. d. d. chem. Gesell.* Jahrg. II. (1869), S. 12.

[2] No distinction is made between cholin and neurin in the latest edition (1883) of Hoppe-Seyler's *Handbuch d. phys.-path. chem. Anal.*

[3] Brieger, *Ber. d. d. chem. Gesell.* Jahrg. XVI. (1883), Sn. 1190, 1406; XVII. Sn. 516, 1137.

[4] *Ann. d. Chem. u. Pharm.* Bd. CXXXIV. (1865), S. 29.

[5] *Jl. of Physiol.* Vol. II. (1879), p. 113. Also in *Zt. f. physiol. Chem.* Bd. III. (1879), S. 260. Gives history and literature of the subject to date.

[6] Baumstark, *Zt. f. physiol. Chem.* Bd. IX. (1885), S. 145.

Cerebrin.[1] $C_{17}H_{33}NO_3$ (?).

Is found in nerves, in pus corpuscles, and largely in the brain. In former times many names were given to the substance when in an impure state, *ex.gr.* cerebric acid, cerebrote, &c. It was first prepared by W. Müller[2] who constructed the above formula from his analysis; the mean of these is C, 68·45. H, 11·2. N, 4·5. O, 15·85. Great doubts are however thrown upon the purity of Müller's preparations by the researches of later observers. From a later investigation it appears to contain less nitrogen than is stated above, the carbon and hydrogen being the same (C, 68·74. H, 10·91. N, 1·44. O, 18·91).[3]

It is prepared from brain substance by extraction with alcohol and purified by recrystallisation from this solvent; its complete separation however from lecithin &c. is difficult, but is attained by treating the mixture with boiling barium hydrate: this, while it has no effect on the cerebrin, decomposes the lecithin.

It is a light, colourless, exceedingly hygroscopic powder, which swells up strongly in water, slowly in the cold, rapidly on heating. When heated to 80° it turns brown, and at a somewhat higher temperature melts, bubbles up, and finally burns away. It is insoluble in cold alcohol, or ether; warm alcohol dissolves it readily. Heated with dilute mineral acids, cerebrin yields a sugar which has recently been shown to be identical with galactose. (See above p. 106.)

Charcot's Crystals.

These remarkable crystals, whose chemical nature and significance have been the subject of much surmise, were first described by Charcot[4] in the spleen and blood of leukhaemic patients. Later researches have confirmed their characteristic appearance in this disease, and have further shown that they occur in health, more particularly in semen, but also in various tissues;[5] they are also found in asthmatic expectorations. They may be readily obtained from semen by extracting with warm water, to which a little ammonia had been added, the residue which remains after semen has been treated with boiling alcohol. The crystals separate out from this solution on concentration, and may be purified by recrystallisation.

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[1] See Gamgee, *Physiol. Chem.* Vol. I. p. 439.

[2] *Ann. d. Chem. u. Pharm.* Bd. CV. (1858), S. 361.

[3] Geoghegan, *Zt. f. physiol. Chem.* Bd. III. (1879), S. 332. See also Parcus, *Jn. f. prakt. Chem.* (N.F.) Bd. XXIV. (1881), S. 310.

[4] *Compt. Rend. Soc. Biol.*, 1853. *Gaz. Hebd.* 1860, p. 755.

[5] Zenker, *Arch. f. klin. Med.* Bd. XVIII. (1876), S. 125. Schreiner, *Ann. d. Chem. u. Pharm.* Bd. 194 (1878), S. 68. Cf. Maly's *Jahresb. über Thierchemie*, 1878, S. 86.

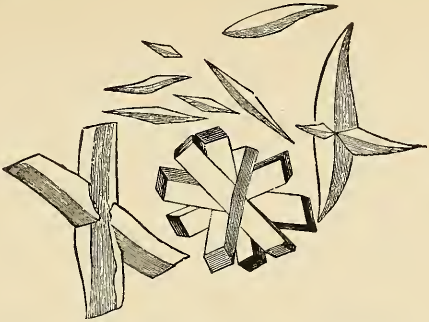

FIG. 6. CHARCOT'S CRYSTALS. (Krukenberg.)

The crystals are insoluble in alcohol, ether, and chloroform, slightly soluble in cold and readily so in hot water. Dilute acids and alkalis also dissolve them readily.

It has been stated that the crystals are in reality a compound of phosphoric acid with a nitrogenous base to which the name spermin[1] has been given, and the formula C_2H_5N(?) has been assigned. This base is obtained by the addition to the crystals of baryta water which forms a phosphate of barium and liberates the base. It is soluble in water and alcohol, yielding strongly alkaline solutions; it may be reconverted into Charcot's crystals by the action of phosphoric acid.[1] This base was at one time regarded as closely related to, if not identical with ethylinimine $C_2H_4 . NH$.[2] It has however been recently shown that the two substances are not identical, and it has further been stated that the composition of spermin is most probably represented by the formula $C_{10}H_{26}N_4$.[3]

AMIDES AND AMIDO-ACIDS. THEIR DERIVATIVES AND ALLIES.

AMIDO-ACIDS OF THE ACETIC SERIES.

1. **Amido-formic acid.** $NH_2 . COOH$.

This substance is identical with carbamic acid, one of the amido-derivatives of carbonic acid, the first acid of the oxalic acid series. It will be described under the oxalic group.

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[1] Schreiner, *loc. cit.*

[2] Ladenburg u. Abel, *Ber. d. d. chem. Gesell.* Jahrg. XXI. (1888), S. 758. Ethylinimine appears (see next ref.) to be nothing but piperazine, Hofman's diethylenediamine.

[3] See Majert u. Schmidt, *Ibid.* Jahrg. XXIV. (1891), S. 241. Poehl, *Ibid.* S. 359.

2. **Glycin.** $C_2H_5NO_2$. [$CH_2(NH_2).COOH$]. (Amido-acetic acid.) (Also called Glycocoll and Glycocine.)

Does not occur in the free state in the animal body, but enters into the composition of several important substances, more especially hippuric and glycocholic acids. It is also a product of the action of hydriodic acid on uric acid, and of boiling acids and caustic alkalis on gelatin: hence the name glycocoll or gelatin-sugar, since it possesses a sweet taste. It crystallises in large, colourless, hard rhombohedra, or four-sided prisms, which are easily soluble in water (1 in 4·3), insoluble in cold, slightly soluble in hot alcohol, insoluble in ether.

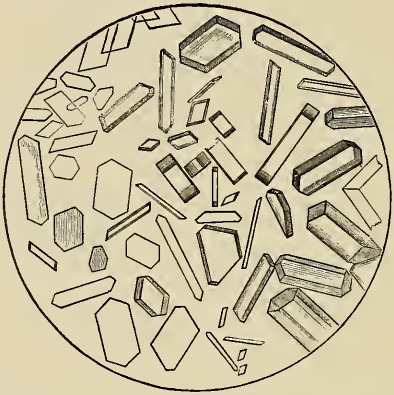

Fig. 7. Glycin Crystals. (After Funke).

Its solutions possess an acid reaction, but a sweet taste. Glycin has also the characteristic property of uniting with both acids and bases, to form crystallisable compounds, as also with salts. In this it exhibits its amidic nature, which is further clearly evidenced by the method of its synthetic production by the action of monochloracetic acid on ammonia:—

$$CH_2(Cl).COOH + 2NH_3 = CH_2(NH_2).COOH + NH_4Cl.$$[1]

Preparation. Either synthetically as above or more usually by the decomposition of hippuric acid by prolonged boiling with hydrochloric acid, whereby it is split up into glycin and benzoic acid, the latter being separated by crystallisation and shaking up with ether in which glycin is insoluble.

3. **Sarkosin.** $C_3H_7NO_2$. [$CH_2.NH(CH_3).COOH$]. (Methyl-glycin.)

Like glycin in its general chemical properties it further resembles it in that it is never found in the free state as a constituent of the animal body. It is however a substance of considerable interest and importance, not merely on account of its chemical relationship to kreatin (see below) but as having been employed in a well-known series of experiments intended to elucidate the probable mode of formation of urea in the body. It was stated that when sarkosin is administered to an animal in quantities such that the nitrogen given as sarkosin is equal to the daily output of nitrogen as urea by the animal, the urea disappears from the urine and is replaced by a new substance.[1] The latter appeared to be a compound of sarkosin and carbamic acid, known generally by the name of methyl-hydantoic acid,—$NH_2 . CO . N(CH_3) . CH_2 . COOH$. This substance may be regarded as arising from the union of one molecule of sarkosin with one of carbamic acid and elimination of one molecule of water, or as being urea in which two atoms of hydrogen are replaced by methyl and a residue of acetic acid respectively:—$NH_2 . CO . N(CH_3)(CH_2 . COOH)$. The conclusions drawn from these observations were that just as methyl-hydantoic acid is supposedly formed by the union of sarkosin with carbamic acid and subsequent dehydration, so also would urea be formed if, instead of sarkosin, ammonia were present, to unite with the carbamic acid, form ammonium carbamate ($NH_4 . NH_2 . CO_2$) and by loss of water yield urea. Subsequent repetition of these ingenious experiments has shown that they are in no way conclusive, for in most cases the sarkosin is largely excreted in an unaltered condition, methyl-hydantoic acid being formed in very minute quantities if at all.[2] It is further interesting to note that the purely chemical reactions which most readily yield methyl-hydantoic acid out of the body, involve the interaction of sarkosin with cyanic compounds such as ammonium or potassium cyanate.[3] Moreover it has been shown that at the temperature of the body sarkosin and urea in solution do not yield methyl-hydantoic acid, although they do in presence of baryta, especially when boiled.[4] These facts show that Schultzen's experiments do not strongly favour the carbamic-acid origin of urea; they further show that the methyl-hydantoic acid is probably not formed by a direct union of sarkosin and urea, and are, from a purely chemical point of view, rather in favour of a cyanic origin of urea.

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[1] Mauthner u. Suida, *Monatshefte f. Chem.* Bd. XI. (1890), S. 373.

4. **Taurin.** $C_2H_7NSO_3$. [$CH_2(NH_2) . CH_2(SO_2 . OH)$]. Amido-ethylsulphonic acid.

Isethionic acid, $CH_2(OH) . CH_2 . SO_2(OH)$, like glycolic acid,

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[1] Schultzen, *Ber. d. d. chem. Gesell.* 1872, S. 578.

[2] Baumann u. von Mering, *Ibid.* 1875, S. 584. E. Salkowski, *Ibid.* S. 638. Also *Zt. f. physiol. Chem.* Bd. IV. (1880), Sn. 55, 101. But see also Schiffer, *Ibid.* Bd. V. (1881), S. 257; Bd. VII. (1883), S. 479.

[3] Baumann u. Hoppe-Seyler, *Ber. d. d. chem. Gesell.* 1874, S. 34. Salkowski, *Ibid.* S. 116.

[4] Baumann u. Hoppe-Seyler, *loc. cit.* Baumann, *Ibid.* S. 237.

$CH_2(OH).COOH$. contains two hydroxyls replaceable by amidogen NH_2, so that two isomeric amido-derivatives can be formed from it. Of these one is amido-isethionic acid $CH_2(OH).CH_2.SO_2(NH_2)$, the other amido-ethylsulphonic acid or taurin.[1]

Taurin is stated to occur in traces in the juices of muscles and of the lungs, but it is known chiefly as a constituent of taurocholic acid, which is one of the characteristic acids of bile, more especially of the carnivora, and above all, of the dog.

It crystallises in colourless, regular, four- or more, usually six-sided prisms; these are readily soluble in water, less so in alcohol. The solutions are neutral. It is a very stable compound, resisting temperatures of less than 240° C; it is not acted on by dilute alkalis and acids, even when boiled with them. It is not precipitated by metallic salts.

Preparation. Ox-bile is boiled for several hours with dilute hydrochloric acid. The fluid residue is separated from the resinous scum, and freed from any remaining traces of bile acids by means of lead acetate, the excess of precipitant being removed by sulphuretted hydrogen. The final filtrate is then concentrated to crystallisation, and the taurin finally purified by recrystallisation from water. The use of the lead salt may be omitted in many cases and the taurin purified by several crystallisations from water.

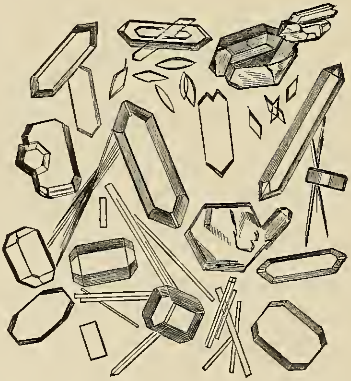

FIG. 8. TAURIN CRYSTALS. (After Kühne.)

The behaviour of taurin when introduced into the alimentary canal is remarkable. In the case of man the larger part reappears in the urine in combination with carbamic acid as tauro-carbamic acid. In dogs a large part is excreted unaltered, together with some tauro-carbamic acid. In herbivora (rabbit) on the other hand a portion of it is excreted in the urine, but the larger part is oxydised, leading to a large increase of sulphates in the urine together with some hyposulphites. Injected subcutaneously it is largely excreted in an unaltered form.[1]

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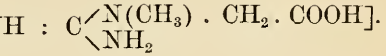
[1] Taurin has usually been regarded as identical with amido-isethionic acid. This is not the case. Seyberth, *Ber. d. d. chem. Gesell.* 1874, S. 391. Erlenmeyer, *Neu. Rep. f. Pharm.* Bd. XXIII. (1874), S. 228.

Tauro-carbamic acid. $NH_2CO . NH(CH_2) . CH_2 . (SO_2OH)$. The remarks which have been already made respecting the nature and formation of sarkosin-carbamic acid apply generally to this acid. It is most easily obtained as a potassium salt by the action of potassium cyanate on taurin.[2]

5. **Kreatin.** $C_4H_9N_3O_2$.



$$[NH : C\begin{matrix}\diagup N(CH_3) . CH_2 . COOH] . \\ \diagdown NH_2 \end{matrix}$$

(Methyl-guanidinacetic acid.)

By the union of ammonia with cyanamide a strongly alkaline base guanidin is obtained: $CN . NH_2 + NH_3 = NH . C(NH_2)_2$ (see below). When sarkosin is employed instead of ammonia a similar reaction takes place, resulting in the formation of kreatin:
$CN.NH_2+CH_2.NH(CH_3).COOH=NH : C(NH_2).N(CH_3).CH_2.COOH$.[3]
Since sarkosin is methyl-amidoacetic acid it is at once obvious that kreatin may be regarded as being methyl-guanidinacetic acid.[4] When cyanamide is treated with boiling baryta water it takes up a molecule of water and yields urea, $CN . (NH_2) + H_2O = CO(NH_2)_2$, hence as might be expected, kreatin yields by similar treatment sarkosin and urea. This is to the physiologist the most important chemical property of kreatin, bearing as it does so closely upon one possible source and mode of formation of urea in the body. (See sub urea.)

Kreatin occurs as a constant and characteristic constituent of muscles and their extracts to an amount which is variable, but may be taken as from 0·2 – 0·3 p. c. on the weight of the muscle.[5] It is also found in nervous tissue, and is said to occur in traces in several fluids of the body. It must however be carefully borne in mind that kreatin very readily loses a molecule of water and thus becomes kreatinin, and that the latter with equal readiness takes up a molecule of water to form kreatin. Hence the kreatin obtained during any analysis need not at all necessarily imply its presence as such in the original tissue or fluid unless due allowance has been made for the possible effect of the methods employed upon the reciprocal conversions of kreatin and kreatinin. This is the cause of the conflicting statements as to the occurrence of kreatin in urine; as a matter of fact this excretion always contains kreatinin. It is on the whole most probable that any kreatin which may be found in urine is due to the conversion of kreatinin into kreatin during its extraction, since it has been shewn [1] that the more rapidly the separation is effected, the less is the quantity of kreatin obtained, and the greater the amount of kreatinin.

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[1] Salkowski, *Ber. d. d. chem. Gesell.* 1872, S. 637. Virchow's *Arch.* Bd. LVIII. (1873); S. 460.

[2] Salkowski, Virchow's *Arch.* Bd. LVIII. (1873), S. 460. *Ber. d. d. chem. Gesell.* 1873, Sn. 744, 1191, 1312. Huppert, *Ibid.* 1278.

[3] Volhard, *Sitzb. d. bayer. Akad.* 1868, Hft. 3, S. 472. Also *Zt. f. Chem.* 1869, S. 318.

[4] Cf. Horbaczewski, *Wien. med. Jahrb.* 1885, S. 459.

[5] Voit, *Zt. f. Biol.* Bd. IV. (1868), S. 77.

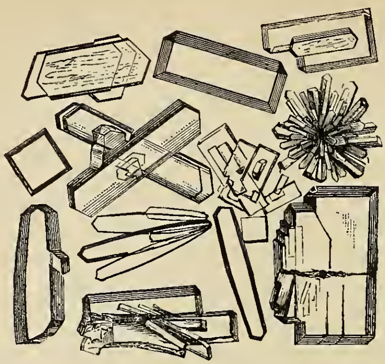

Fig. 9. Kreatin Crystals. (Krukenberg after Kühne.)

In the anhydrous form kreatin is white and opaque, but crystallises with one molecule of water in colourless transparent rhombic prisms.

The crystals are soluble in 75 parts of cold water, extremely soluble in hot; slightly soluble in absolute alcohol, they are more soluble in dilute spirit and are insoluble in ether. The aqueous solutions are neutral in reaction.

Kreatin is a very weak base, scarcely neutralising the weakest acids, with which it forms soluble crystalline compounds.

Preparation. Most conveniently from 'Liebig's Extract.' This is dissolved in 20 parts of water and precipitated by a slight excess of basic acetate of lead. The filtrate is then freed from the lead salt by means of sulphuretted hydrogen and concentrated at moderate temperature (avoid boiling) to a thin syrup. On standing in a cool place for two or three days the kreatin crystallises out. The crystals are removed by filtration, washed with 88 p. c. alcohol, and purified by recrystallisation from water.[2]

Kreatin yields no very striking reactions by means of which it can readily be identified. It reduces Fehling's fluid by prolonged boiling without any separation of cuprous oxide. On boiling in presence of alkaline mercuric oxide, a transient red colour is obtained and finally a separation of metallic mercury. The reactions of kreatinin on the other hand are striking (see below), and hence kreatin may be identified with most certainty by conversion into kreatinin, and the determination of the presence of the latter substance. The conversion is readily effected by boiling with dilute mineral acids, during which process kreatin loses one molecule of water: $C_4H_9N_3O_2 = C_4H_7N_3O + H_2O$.

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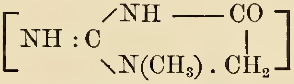
[1] Dessaignes, *Jn. de Pharm. et Chim.* (3) T. xxxii. (1857), p. 41.

[2] The mother-liquor from the kreatin may be used for the preparation of hypoxanthin and sarcolactic acid. Drechsel, *Darstell. physiol.-chem. Präparate,* 1889, S. 29.

Mention has already been made of the possible and very probable genetic relationship of urea to muscle-kreatin (see § 484). This is a question to which brief reference will again be made under urea.

6. **Kreatinin.** $C_4H_7N_3O$.



$$\left[\mathrm{NH:C}\begin{array}{ll}\diagup\mathrm{NH} \text{——} & \mathrm{CO} \\ & \;| \\ \diagdown\mathrm{N(CH_3)}\,. & \mathrm{CH_2}\end{array}\right]$$

Kreatinin as already stated is simply a dehydrated form of kreatin. It occurs normally as a constant constituent of urine, varying however in amount from 0·5 to 4·9 grm. per diem according to the amount of proteid food (meat) eaten.[1] It is not a normal constituent of mammalian muscle but is found in the muscles of some fishes,[2] and has been obtained from sweat.[3] It crystallises in colourless prisms or tables according to the conditions under which the separation takes place and the mode of preparation, and frequently, owing to imperfect development, the crystals assume a very characteristic 'whetstone' form.

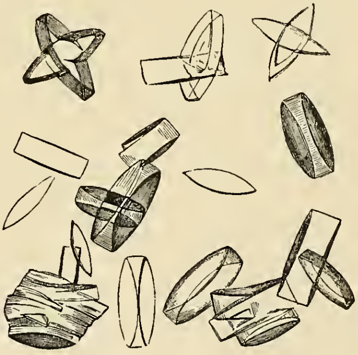


Fig. 10. Kreatinin Crystals. (Krukenberg after Kühne.)

Kreatinin is readily soluble in cold water (1 in 11·5), also in alcohol, but is scarcely soluble in ether. The aqueous solutions are usually alkaline, but some observers regard the alkalinity as due to impurities.[4] It acts as a powerful base, forming compounds with acids and salts which crystallise well. Of these the most important is the salt with chloride of zinc $(C_4H_7N_3O)_2ZnCl_2$, both on account of its characteristic crystalline form and of its general insolubility in comparison with the other compounds of this substance. Hence its formation is employed not merely for the determination of kreatinin but for its separation from solutions. It crystallises in warty lumps composed of aggregated masses of prisms, or fine needles.

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[1] Voit, *loc. cit.* (sub kreatin).

[2] Krukenberg, *Unters. physiol. Inst.* Heidelb. Bd. IV. Hf. 1. (1881), S. 33.

[3] Capranica, *Bull. R. Accad. med. Roma*, Ann. VIII. (1882), No. 6.

[4] Salkowski, *Zt. f. physiol. Chem.* Bde. IV. (1880), S. 133; XII. (1888), S. 211.

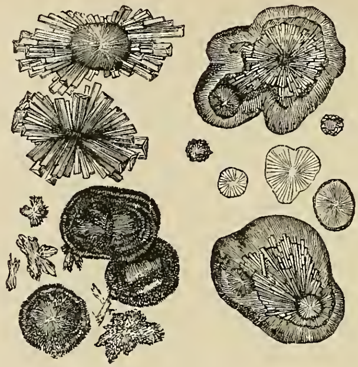

FIG. 11. KREATININ-ZINC-CHLORIDE CRYSTALS. (Krukenberg after Kühne.)

This compound is formed when a concentrated *neutral* solution of the zinc salt is added to a not too dilute solution of kreatinin, and since it is almost insoluble in alcohol it is frequently convenient to employ alcoholic rather than aqueous solutions of the two substances.

Preparation.[1] This does not admit of any useful brief description, but the principles involved are the following:—

(i) By the action of dilute boiling mineral acids on kreatin.

(ii) By concentrating large volumes of urine to a small bulk. From this the kreatinin is obtained as a compound either by the addition of chloride of zinc or by precipitation with mercuric chloride. From these compounds it is then separated by boiling with hydrated oxide of lead, and is finally purified by crystallisation.

It may also be precipitated by phospho-tungstic and phospho-molybdic acids.[2]

Apart from the characteristic formation of the compound with zinc chloride, kreatinin yields several well-marked reactions, of which the following are the more striking.

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[1] For details see Hoppe-Seyler, *Phys.-path. chem. Anal.* 1883, S. 182, and Neubauer u. Vogel, *Harn-analyse*, 1890, S. 228.

[2] For recent synthesis see Horbaczewski, *loc. cit.* (sub kreatin).

1. *Weyl's reaction.*[1] To the suspected solution a few drops of very dilute sodium nitro-prusside [$Na_2(NO)FeCy_5$] are added, and then, drop by drop, some dilute caustic soda. If kreatinin is present a fine but transient ruby-red colour is obtained which speedily passes into yellow. If the solution is now acidulated with acetic acid and warmed it turns at first greenish and finally blue.[2] This last colour is due to the formation of Prussian-blue.[3] Weyl's reaction is extremely delicate and suffices to detect ·0287 p. c. of kreatinin in pure solution, or ·066 p. c. in urine. According to Krukenberg the reaction is best obtained by adding the caustic soda first and then a few drops of concentrated solution of the nitro-prusside. Guareschi recommends the use of 10 p. c. solutions of the respective reagents.[4]

When applied to urine the absence of acetone should be ascertained, since it also gives a similar ruby-red colour, but no subsequent blue can be obtained from it, and the solution when yellow turns red again on the addition of strong acetic acid. Hydantoin or methyl-hydantoin also yields the red colouration.

2. *Jaffe's reaction.*[5] On the addition of an aqueous solution of picric acid and a few drops of dilute caustic soda an intense red colouration is produced. This suffices to detect ·1 part of kreatinin in 5000 of water. Acetone alone gives a similar colouration, but to a comparatively very feeble extent.

By prolonged boiling of kreatinin with Fehling's fluid, reduction takes place, but there is no simultaneous separation of cuprous oxide, and it appears that kreatinin may prevent the separation of the oxide when the reduction is due not to itself but to such a substance as dextrose.[6]

7. **Leucin.** $C_6H_{13}NO_2$. [CH_3 . $(CH_2)_3CH(NH_2)COOH$]. (*a*-Amido-caproic acid.)

Is a characteristic product of the decomposition of proteids and gelatin whether by the action of boiling acids, caustic alkalis, or putrefactive influences. It occurs normally in variable amounts in the pancreas, spleen, thymus, thyroid, salivary glands, liver, &c., and also in plants, more especially in those parts in which reserve materials are accumulated, such as bulbs, tubers, and seeds. It is also typically formed during the tryptic (pancreatic) digestion of proteids to an extent which amounts on the average to some 8—10 p. c. on the proteid digested, and is in this case always accompanied by tyrosin. It may occur in the urine, more particularly in cases of acute yellow atrophy of the liver; but its presence in this excretion in other and more general diseased conditions of the liver is by no means so constant or certain as it presumably would be on the common assumption that a large part of the urea leaving the body is due to its formation from leucin under the converting action of the liver.[1]

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[1] *Ber. d. d. chem. Gesell.* 1878, S. 2175.

[2] Salkowski, *Zt. f. physiol. Chem.* Bde. IV. (1880), S. 133; IX. (1885), S. 127.

[3] Krukenberg, *Verhand. d. phys.-med. Ges.* Würzburg, Bd. XVIII. (1884), S. 5. Confirmed by Salkowski. Cf. Colasanti, Moleschott's *Unters.* Bd. XIII. (1888), Hf. 6.

[4] *Ann. di chim. e di farm.* Ser. 4 T. v. (1887), p. 195.

[5] *Zt. f. physiol. Chem.* Bd. x. (1886), S. 399.

[6] Worm Müller, Pflüger's *Arch.* Bd. XXVII. (1882), S. 59.

As usually obtained in a more or less impure form it crystallises in rounded fatty-looking lumps which are often collected together and sometimes exhibit radiating striation. When pure, it forms very thin, white, glittering flat crystals. It is extremely soluble in hot water, less so but still very soluble in cold water, soluble in alcohol, insoluble in ether. The crystals feel oily to the touch, and are without smell and taste. Leucin is particularly soluble in presence of acids and alkalis. The aqueous solutions are lævorotatory, acid and alkaline solutions on the other hand dextrorotatory.

Preparation. (i) From horn shavings by prolonged boiling with sulphuric acid, 5 of acid to 13 of water. The resulting fluid is neutralised by baryta and filtered, the excess of baryta removed by the cautious addition of dilute sulphuric acid, and the final filtrate concentrated to crystallisation. It is separated from tyrosin by repeated crystallisation, taking advantage of the great solubility of leucin and the slight solubility of tyrosin. (ii) From the products of the tryptic (pancreatic) digestion of proteids. After *prolonged* digestion, using thymol and salicylic acid to prevent putrefaction, the fluid is filtered, moderately concentrated, and set aside to crystallise; by this means a large part

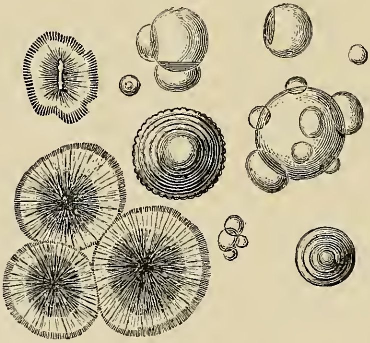

FIG. 12. LEUCIN CRYSTALS. (Krukenberg.)

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[1] Cf. Salkowski, *Die Lehre vom Harn*, 1882, S. 427. Lea, *Jl. of Physiol.* Vol. xi. (1890), p. 258.

of the accompanying tyrosin is removed. The filtrate is now further concentrated, treated with excess of *hot* alcohol, which precipitates the peptones, and filtered while hot. If much leucin is present a large part of it crystallises out on cooling the alcoholic filtrate, and the rest on concentrating by slow evaporation. There is a large loss of leucin by both the above methods, and the resulting product is far from pure. To obtain pure leucin it should be synthetised by the action of ammonia on α-bromcaproic acid.[1]

Even an approximately quantitative separation of leucin from solutions where it is mixed with other substances, e. g. an extract of tissues or a digestive mixture, is a matter of great difficulty. Advantage may in some cases be taken of its behaviour towards hydrated oxide of copper, with which it forms a compound.[2]

For ordinary practical purposes the microscopic appearance of the crystals affords the most convenient means for recognising leucin, and in this way very minute traces may be determined with certainty. The confirmation of the clue thus afforded by the application of chemical tests is however not easy unless a fair amount of material is at hand, and that in a pure condition. In the latter case the following tests may be applied. (i) When carefully heated to 170° leucin sublimes and yields a characteristic odour of amylamin. The only other substance of physiological importance ordinarily met with which yields a sublimate on heating is hippuric acid, due to its decomposition and the sublimation of the benzoic acid thus set free. (ii) *Scherer's test.* Only applicable to very pure leucin. The suspected substance is evaporated carefully to dryness with nitric acid on the lid of a platinum crucible; the residue, if it is leucin, will be almost transparent and turn yellow or brown on the addition of caustic soda. If this be again very carefully concentrated with the alkali an oily drop is obtained, which runs over the platinum in a spheroidal state.

The optical properties of leucin have not as yet been fully worked out. Experiment shows that its solutions are sometimes optically active, at other times inactive, dependently upon the source and mode of formation of the leucin. This corresponds to the expectations as to its optical behaviour based, in accordance with the Van't Hoff-Le Bel hypothesis, upon its constitutional formula.[3]

The possible relationship of leucin to the formation of urea in the body has been already pointed out (§ 488). It will be further considered under urea.

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[1] Hüfner, *Jn. f. prakt. Chem.* (2) Bd. I. (1870), S. 6.

[2] Hlasiwetz u. Habermann, *Ann. d. Chem. u. Pharm.* Bd. CLXIX. (1873), S. 150.

[3] For details see Mauthner, *Zt. f. physiol. Chem.* Bd. VII. (1882–83), S. 222. Schulze, *Ibid.* Bd. IX. (1885), S. 100. Lewkowitsch, *Ber. d. d. chem. Gesell.* 1884, S. 1439. Lippmann, *Ibid.* S. 2835. Schulze u. Bosshard, *Zt. f. physiol. Chem.* Bd. X. (1886), S. 134.

AMIDO-ACIDS OF THE LACTIC SERIES.

Cystin. $(C_3H_6NSO_2)_2 \, . \, [S \, . \, C(CH_3)(NH_2) \, . \, COOH]_2$. Amido-sulpholactic acid.[1]

Is the chief constituent of a rarely occurring urinary calculus in men and dogs. It may also occur in renal concretions, and in gravel, and is occasionally found in urine, from which it separates out as a greyish sediment on standing. It is prepared from this sediment, or better still from cystic calculi, by solution in ammonia. This solution is then allowed to evaporate spontaneously and yields the cystin in regular, colourless, six-sided tables of very characteristic appearance. Cystin may be separated from urine by taking advantage of the formation of a sodium salt of benzoyl-cystin when it is shaken up with a few drops of benzoyl-chloride.[2]

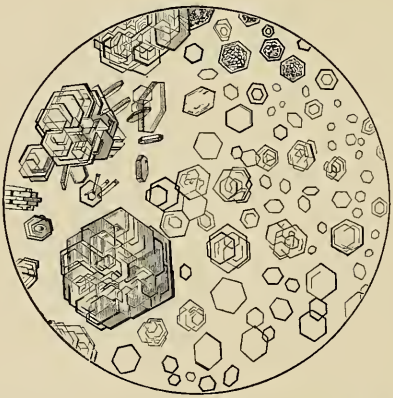

FIG. 13. CYSTIN CRYSTALS. (After Funke.)

Cystin is insoluble in either water, alcohol, or ether, readily soluble in ammonia, differing in this respect from uric acid, also in many alkaline carbonates and in mineral acids. Its solutions are strongly lævorotatory, $(a)_D = -205{\cdot}9°$ in hydrochloric acid 11·2 p. c.[1] or if the acid is dilute $(a)_D = -214°$.[2]

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[1] The constitution of cystin has been variously stated by different authors, and will only be known with certainty when its synthesis has been accomplished. Slightly different formulæ have been assigned to it, containing respectively 5, 6, and 7 atoms of hydrogen. The literature is fully quoted by Külz, *Zt. f. Biol.* Bd. xx. (1884), S. 1. Cf. Baumann, *Zt. f. physiol. Chem.* Bd. VIII. (1884), S. 299.

[2] Goldmann u. Baumann, *Zt. f. physiol. Chem.* Bd. XII. (1888), S. 254. Udránsky u. Baumann, *Ibid.* Bd. xv. (1891), S. 87.

Apart from the characteristic crystalline form and its solubility in ammonia, the fact that cystin is one of the few crystalline substances, occurring physiologically, which contain sulphur, renders its detection very easy. Thus when boiled with caustic alkalis a sulphide of the alkali is obtained which gives a dark stain on silver foil; also a brown or black colouration appears when cystin is boiled in a test-tube with a solution of oxide of lead in caustic soda.[3]

AMIDO-ACIDS OF THE OXALIC SERIES.

1. **Carbamic acid.** $NH_2(COOH)$.

Carbonic acid is more usually classed at the head of the acids of the glycolic (lactic) series. It exhibits however a remarkable difference from the remaining acids of this group, since they are all monobasic, whereas carbonic acid is dibasic. It may therefore be more appropriately classed with the dibasic acids of the oxalic series. In virtue of the two replaceable hydroxyls which it contains, it yields two amido-derivatives, of which the first is carbamic acid, the second urea $(NH_2)_2CO$ or carbamide. Carbamic acid is a substance of peculiar interest to the physiologist on account of the important part it is frequently supposed to play in the formation of urea in the animal body. It is formed by the direct union of equal molecules of *dry* ammonia and carbonic anhydride, a second molecule of ammonia uniting with it at the same time to yield the ammonium salt or ammonium carbamate. Thus $2NH_3 + CO_2 = NH_4\,NH_2CO_2$: simple dehydration of this salt yields urea $(NH_2)_2CO$. This point will be returned to further on when discussing the probable mode of formation of urea in the body.

Carbamic acid is unknown in the free state; its best known salt is that with ammonium, but many others have been prepared. It further appears that some of its salts occur in serum, and it is also stated to be formed during the oxidation of glycin, leucin, and tyrosin by means of potassium permanganate in alkaline solution.[4] Ammonium carbamate is extremely soluble in water, in which solution it is gradually converted into the carbonate. At ordinary pressures when heated to 60° it is decomposed into ammonia and carbonic anhydride, but under pressure at 130°–140° it yields urea. When electrolysed in cold aqueous solution by a rapidly and continuously commutated current the salt similarly loses water and yields urea (Drechsel). The dehydration may be represented as taking place in the following way:—

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[1] Mauthner, *Zt. f. physiol. Chem.* Bd. VII. (1883), S. 225. Cf. Drechsel, *Arch. f. Physiol.* Jahrg. 1891, S. 247.

[2] Baumann, *loc. cit.* S. 303.

[3] The following literature may be additionally consulted on the occurrence of cystin in urine. *Zt. f. physiol. Chem.* Bde. IX. 129, 260; XII. 254; XIV. (1889), 109. Virchow's *Arch.* Bd. C. (1885), S. 416. Maly's *Jahresb.* 1886, S. 465. *Berl. klin. Wochensch.* 1889, No. 16. *Zt. f. klin. Med.* Bd. XVI. (1889), S. 325.

[4] Drechsel, *Ber. d. k. s. Gesell. d. Wiss.* Leipzig. *Math. naturwiss. Cl.* Juli, 1875. *Jn. f. prakt. Chem.* (2) Bd. XII. (1875), S. 417; XVI. (1877), S. 180; XXII. (1880), S. 476. *Arch. f. Physiol.* Jahrg. 1880, S. 550. But see also Hofmeister, Pflüger's *Arch.* Bd. XII. (1876), S. 337.

i. $NH_2 . CO . O . NH_4 + O = NH_2 . CO . O . NH_2 + H_2O.$

ii. $NH_2 . CO . O . NH_2 + H_2 = NH_2 . CO . NH_2 + H_2O;$

or by the action first of H_2 and then of O.[1]

2. **Aspartic** (*or asparaginic*) **acid.** $C_4H_7NO_4$. [COOH . CH_2 . CH (NH_2) . COOH]. Amido-succinic acid.

This acid is chiefly obtained from plant extracts, and occurs notably in beet-sugar molasses. It may be synthetised, but is most conveniently prepared by boiling asparagin with caustic alkalis or mineral acids. It is also a typical product of the action of boiling mineral acids and caustic baryta on both vegetable and animal proteids (*antea* p. 49) and of acids on gelatin,[2] being usually accompanied by its homologue, glutamic acid. It is also now recognised as a product in minute quantities of the pancreatic digestion of fibrin[3] and vegetable glutin,[4] although it does not occur as a constituent of any animal tissue or secretion. It crystallises in rhombic prisms which are but sparingly soluble in cold water or alcohol, but readily soluble in boiling water. Its solutions, if strongly acid, are dextrorotatory, but if alkaline, lævorotatory. It forms a characteristic readily crystallisable compound with oxide of copper, which is practically insoluble in cold, but soluble in boiling water, and may be used for the separation of aspartic acid from solutions in which it is mixed with other substances.[5]

3. **Glutamic** (*or glutaminic*) **acid.** $C_5H_9NO_4$. (Amido-pyrotartaric acid).

This acid is homologous with aspartic acid. The circumstances and conditions under which it occurs are in general the same as for aspartic acid, but it has not as yet been obtained by the action of pancreatic enzymes on proteids and is never found in any animal tissues or secretions. But as a product, often to a large amount, of the artificial decomposition of proteids it acquires some considerable importance. It is always prepared by treating proteids with boiling mineral acids.[1]

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[1] Cf. Ludwig's *Festschrift*, 1887, S. 1.

[2] Horbaczewski, *Sitzb. d. k. Akad. d. Wiss. Wien.* Bd. LXXX. (2 Abth.) Juni-Hft. 1880.

[3] Radziejewski u. Salkowski, *Ber. d. deutsch. chem. Gesell.* Jahrg. VII. (1874), S. 1050.

[4] v. Knieriem, *Zeitsch. f. Biol.* Bd. XI. (1875), S. 198.

[5] Hofmeister, *Sitzb. d. k. Akad. d. Wiss. Wien,* Bd. LXXV. (1877), 2 Abth. März-Hft.

It crystallises in rhombic tetrahedra or octahedra; is not very soluble in cold, but readily soluble in hot water; insoluble in alcohol and in ether. Its aqueous and acid solutions possess a strong dextrorotatory power.

4. **Asparagin.** $C_4H_8N_2O_3 + H_2O$. [$COOH . CH_2 . CH (NH_2) . CONH_2$]. Amido-succinamic acid.

Although asparagin is not found as a constituent of the animal body it is a substance of considerable interest to the physiologist. Not only is it closely related to aspartic acid, into which it may be converted by the action of boiling acids and alkalis, yielding at the same time ammonia, but it undoubtedly plays a most important part in the constructive proteid metabolism of plants. Further it exists in not inconsiderable amount in many plant-tissues used as food by man, and is known, like so many of the members of the numerous class of amido-acids to which it belongs (leucin, glycin, &c.) to give rise to urea when taken into the body of carnivora,[2] and to uric acid in that of birds.[3]

In plants asparagin, like leucin, is found chiefly in those parts which afford a store of reserve material, such as bulbs, tubers, &c., and the cotyledons of seeds. The amount is however largely increased during germination, and it is therefore present in frequently very large quantities in seedlings, as for instance those of yellow lupins (30 p.c.). The increase in the young growing plant is most probably due chiefly to a formation of asparagin out of the decomposition of reserve-proteids, although some may be formed synthetically. The amount is greatest when the seeds are germinated in the dark and the seedling subsequently grown for some time in semi-obscurity and shielded from the access of carbonic anhydride. Under these conditions the formation of non-nitrogenous (? carbohydrate) material is simultaneously prevented; and putting the two facts together it appears probable that the disappearance of asparagin in seedlings grown under ordinary conditions is due to its consumption for the synthetic production of proteids.[4] It is conceivable that the amido-acids and amides may similarly play some part in the synthetic metabolism of animal tissues, though to a presumably much slighter extent, bearing in mind how in plants constructive metabolism preponderates so largely over the destructive.[5]

Asparagin crystallises readily in large rhombic prisms which are not very soluble in cold, but readily soluble in hot water, and are insoluble in absolute alcohol and in ether. Its solutions are dextrorotatory. It may be prepared synthetically,[1] but is usually obtained by crystallisation from the expressed juice or extracts of the seedlings of peas, beans, or lupins.[2] Mercuric nitrate yields a precipitate with asparagin which may be used for its separation from vegetable extracts.[3] Urea-ferment converts it into succinic acid.[4]

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[1] Ritthausen u. Kreusler, *Jn. f. prakt. Chem.* (2) Bd. III. (1871), S. 314.

[2] v. Knieriem, *Zt. f. Biol.* Bd. x. (1874), S. 277.

[3] v. Knieriem, *Ibid.* XIII. (1877), S. 36.

[4] Cf. Vines, *Physiology of Plants*, pp. 124, 150, 174.

[5] Lea, *Jl. of Physiol.* Vol. XI. (1890), p. 258.

One point of interest with respect to asparagin remains to be briefly mentioned. Seeing that in plants the nitrogen requisite for the construction of proteids appears to be obtained largely from asparagin, is there any evidence that in animals also the nitrogen of this substance can take the place of that of proteids? The answer to this question may be stated as follows: When asparagin is administered to carnivora or birds practically the whole of it is converted into urea or uric acid respectively.[5] Thus in carnivora at least there is no diminution of proteid metabolism, such as is observed under a gelatin diet, when asparagin is added to the food. In herbivora on the other hand there appears to be somewhat distinct evidence that a part of the nitrogen in proteids may be replaced by that of asparagin.[6]

The question as to the importance of the nitrogen of asparagin as a possible replacer of that of proteids arose first in connection with the dispute already referred to (p. 122) on the mode of formation of fats in the animal body. In the experiments of Weiske and Wildt[7] on which Voit chiefly based his original views, a diet of potatoes was largely used. The amount of proteid in these was calculated from the total nitrogen they contained, on the assumption that there was no nitrogen present in them in any form other than that of proteids. As a matter of fact potatoes contain a not inconsiderable quantity of asparagin,[8] so that making allowance for this the total amount of proteid given in their experiments was much less than they supposed, and might not have sufficed to account for the fat stored up. This difficulty would obviously be got over if it could be shown that the nitrogen of asparagin can play the part of the nitrogen of proteids.

[1] See recently Piutti, *Chem. Centralb.* Bd. xix. (1888), S. 1459.

[2] Piria, *Ann. de Chim. et de Phys.* (3) T. xxii. (1847), p. 160. Schulze u. Bosshard, *Zt. f. physiol. Chem.* Bd. ix. (1885), S. 420.

[3] Schulze, E., *Ber. d. d. chem. Gesell.* 1882, S. 2855.

[4] Bufalini, *Ann. di chim. e di farmac.* (4) T. x. (1889), p. 207.

[5] Von Knieriem, *loc. cit.* But cf. von Longo, *Zt. f. physiol. Chem.* Bd. i. (1877), S. 213.

[6] Weiske, *Zt. f. Biol.* Bd. xx. (1884), S. 277. Weyl, *Biol. Centralb.* Bd. ii. (1882–83), S. 277. These give copious references to literature up to date. In addition see Voit, *Sitz. d. Bayr. Akad.* 1883, S. 401. Röhmann, Pflüger's *Arch.* Bd. xxxix. (1886), S. 21. (On storage of glycogen.)

[7] *Zt. f. Biol.* Bd. x. (1874), S. 1.

[8] Schulze u. Barbieri, *Landwirth. Versuchs-Stat.* Bd. xxi. (1877), S. 63.

THE UREA AND URIC ACID GROUP.[1]

1. **Urea.** $(NH_2)_2CO$. (*Carbamide*).

This is the chief nitrogenous constituent of normal urine in mammalia and some other animals. The urine of birds also contains a small amount, more particularly on a meat diet. Average normal human urine contains from 2·5 — 3·2 p.c., the average total daily excretion varying from 22—35 grams or as a mean 30 grams. It is also found in minute quantities in normal blood [2] (·025 p.c.) serous fluids, lymph, and aqueous humour: it is not usually met with in the tissues except that of the liver.[3] It is never present in normal mammalian muscles, but may make its appearance there under certain pathological conditions. Under ordinary conditions the amount of urea in sweat is almost inappreciable, but the older statements of its occurrence in this excretion have recently received confirmation, and it appears that this source of nitrogenous loss to the body may have to be taken into account.[4]

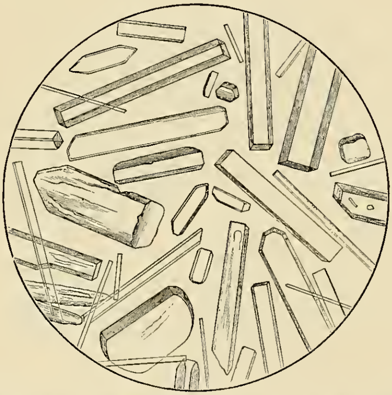

FIG. 14. UREA CRYSTALS SEPARATED BY SLOW EVAPORATION FROM AQUEOUS SOLUTION. (After Funke.)

When pure it crystallises from a concentrated solution in the form of long, thin glittering needles. If deposited slowly from dilute solutions, the form is that of four-sided prisms with pyramidal ends; these are always anhydrous. When the separation occurs rapidly, as for instance from a strong alcoholic solution on a glass-slide, the typical crystalline form is not readily observed, but rather that of irregular dendritic crystals.

Urea is very soluble in cold water, distinctly less soluble in cold alcohol, readily so in hot; it is insoluble in anhydrous ether and in petroleum-ether.[1] It possesses a somewhat bitter, cooling taste, resembling saltpetre.

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[1] For full details of the reactions, properties, and methods of determining and dealing practically with the members of this group, consult in all cases Neubauer u. Vogel, *Analyse des Harns.* Salkowski u. Leube, *Die Lehre vom Harn.* Hoppe-Seyler, *Physiol.-path. chem. Analyse.*

[2] Gscheidlen, *Stud. über d. Ursprung d. Harnstoffs*, Leipzig, 1871.

[3] But see Hoppe-Seyler, *Zt. f. physiol. Chem.* Bd. v. (1881), S. 348.

[4] Argutinsky, Pflüger's *Arch.* Bd. XLVI. (1890), S. 594.

Preparation. (i) From urine by concentration to a sirupy state, extraction of the residue with absolute alcohol, and concentration of the alcoholic extract, by slow spontaneous evaporation in a warm place, until the urea crystallises out. This is then purified by recrystallising from alcohol, decolourising with charcoal if required. Or the urea may be precipitated as nitrate by the addition of *pure colourless* nitric acid to strongly concentrated urine cooled to 0°. The nitrate is then decomposed in water by the addition of barium carbonate, and the urea extracted as before with alcohol. (ii) Synthetically in many ways, of which the most usual and convenient is by mixing equivalent proportions of ammonium sulphate and potassium cyanate; the ammonium cyanate thus formed is evaporated to dryness, whereupon it undergoes a molecular transformation to urea, which is then extracted with alcohol: thus $NH_4 . CON = NH_2 . CO . NH_2$.

It is interesting to note that the above synthesis of urea, obtained in 1828 by Wöhler, was the first instance in which a substance ordinarily elaborated by the specific activity of the animal body was artifically prepared.

Urea readily forms compounds with acids and bases; of these the following are important as a means of detection and identification.

Nitrate of urea. $(NH_2)_2 CO . HNO_3$.

Obtained by the addition of a slight excess of *pure colourless* nitric acid to a moderately concentrated solution of urea. The nitrate should separate out rapidly in the form of six-sided or rhombic tables, frequently aggregated in piles, but the successful obtaining of typical crystals requires some attention to the concentration of the solution.

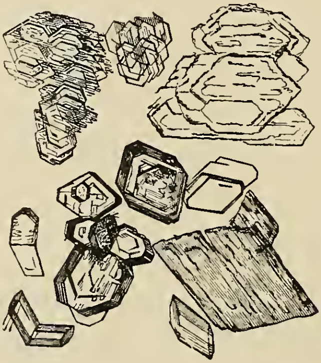

FIG. 15. CRYSTALS OF NITRATE OF UREA. (Krukenberg after Kühne.)

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[1] Petroleum-ether consists of the products, with low boiling-points (up to 120°), of the distillation of ordinary petroleum. It is also known commercially under the name of ligroin.

The crystals are but slightly soluble in nitric acid, or alcohol, more soluble in cold water, and much more so in hot water. They are insoluble in ether.

Oxalate of urea. $[(NH_2)_2CO]_2 . H_2C_2O_4 + H_2O$.

Obtained by the addition of concentrated aqueous solution of oxalic acid to a concentrated aqueous solution of urea. This salt crystallises out in rhombic tables closely resembling those of the nitrate, but they are frequently aggregated into a characteristic prismatic form. As in the case of the nitrate some care is required with respect to the concentration of the respective solutions during its preparation.

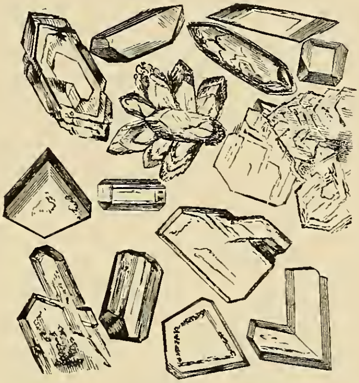

FIG. 16. CRYSTALS OF OXALATE OF UREA. (Krukenberg after Kühne.)

The crystals are less soluble in oxalic acid than in water, but may in other respects be taken as resembling those of the nitrate in respect of their solubilities.

Of the many salts which urea forms with other bases and salts those which it yields with mercuric oxide and nitric acid are of most importance. When a solution of mercuric nitrate is added to one of urea a precipitate is formed which, dependently upon the concentration and relative amounts of the two solutions, may contain some one of three possible salts, consisting of $[(NH_2)_2 CO]_2 . Hg (NO_3)_2$ united with 1, 2, or 3 molecules of mercuric oxide (HgO). When the solutions are fairly neutral and *dilute*, the salt with 3 molecules of HgO is formed $[(NH_2)_2 CO]_2 . Hg(NO_3)_2 . 3 HgO$. This is the salt formed in the reactions on which Liebig's volumetric method for the determination of urea is based.

The other more important reactions of Urea.

1. Urea may be heated dry in a tube to 120° without being decomposed; on further raising the temperature it melts at 132·6°[1]

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[1] Reissert, *Ber. d. d. chem. Gesell.* Bd. XXIII. (1890), S. 2244.

and afterwards gives off ammonia, and if heated to 150° for some time is converted largely into biuret: $2(NH_2)_2CO = NH_2.CO.NH.CO(NH_2) + NH_3$. On further heating to a higher temperature (200°) it is largely converted into cyanuric acid. When biuret is dissolved in water and treated with caustic soda and dilute sulphate of copper it yields the well-known pink colour employed for the detection of peptones, and hence called the 'biuret reaction.' In the application of the test to urea some caution is requisite while heating the suspected substance to avoid carrying the decomposition beyond the biuret stage. When boiled in aqueous solution with strong sulphuric acid or alkalis it is gradually decomposed, under assumption of two molecules of water, into carbonic acid and ammonia; the same decomposition ensues by simple heating of the aqueous solution in sealed tubes, to 180°. This forms the basis for the older 'Bunsen method' of estimating urea. A similar change (hydration) is produced under the influence of several micro-organisms which are found in urine undergoing alkaline fermentation. Of these the best known is the Micrococcus ureae[1] from which a soluble hydrolytic enzyme may be extracted.[2] (See above, p. 70.)

2. When treated with nitrous acid, e.g. impure yellow nitric acid, it is decomposed finally into carbonic anhydride, nitrogen, and water: $(NH_2)_2CO + 2HNO_2 = CO_2 + 2N_2 + 3H_2O$. A similar decomposition is obtained by the action of sodium hypochlorite or hypobromite: $(NH_2)_2CO + 3NaBrO = 3NaBr + CO_2 + N_2 + 2H_2O$. Since the volume of nitrogen evolved is constant for a given weight of urea, this latter reaction forms the basis of a method for the quantitative determination of urea. (Knop-Hüfner.)

3. When a crystal of urea is treated with a drop of concentrated freshly prepared aqueous solution of furfurol — $C_5H_4O_2$ (aldehyde of pyromucic acid) and then *immediately* with a drop of hydrochloric acid (sp. gr. = 1·10) a play of colours is observed which passes rapidly from yellow through green, blue, and violet to a final brilliant purple. The test may be also applied by the addition of three drops of the acid to a mixture of one drop of 1 p.c. aqueous urea solution and ·5 cc. of aqueous furfurol solution.[3]

Detection in Solutions. In addition to the microscopic appearance of the crystals obtained on evaporation, the nitrate and oxalate should be formed and examined. Another part should give a precipitate with mercuric nitrate, in the absence of sodium chloride but not in the presence of this last salt if in excess; in presence of sodium chloride the mercuric nitrate reacts first with the sodium salt in preference to the urea. A third portion is treated with nitric acid containing nitrous fumes; if urea is present, nitrogen and carbonic acid will be obtained. To a fourth part pure nitric acid in excess and a little mercury are added, and the mixture is warmed. In presence of urea a *colourless* mixture of gases (N and CO_2) is given off. A fifth portion is treated, after evaporation to dryness, in the way above described for the application of the biuret reaction, and a sixth part is tested with furfurol.

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[1] Pasteur, *Compt. Rend.* T. L. (1860), p. 869. Van Tieghem, *Ibid.* T. LVIII. (1864), p. 210. Jaksch, *Zt. f. physiol. Chem.* Bd. V. (1881), S. 395.

[2] Musculus, Pflüger's *Arch.* Bd. XII. (1876), S. 214. Lea, *Jl. of Physiol.* Vol. VI. (1885), S. 136.

[3] Schiff, *Ber. d. d. chem. Gesell.* 1877, S. 773.

Quantitative determination. The methods are based on some of the reactions above described. They consist of (i) Precipitation by a standardised solution of mercuric nitrate (Liebig). (ii) Decomposition into carbonic acid and nitrogen by means of sodium hypobromite, and measurement of the *volume* of nitrogen (Knop-Hüfner). (iii) Conversion into carbonic acid and ammonia by heating in a sealed tube with an ammoniacal solution of barium chloride, and determination of the *weight* of barium carbonate obtained. (Bunsen.)

Although simple in principle, the above methods, and especially the first, require the careful observance of certain precautions to ensure accuracy. The needful precautions have recently been most assiduously investigated, more particularly by Pflüger and his pupils, and of these and of the application of the methods a full account is given in Neubauer and Vogel's exhaustive work *Die Analyse des Harns.*

The determination of the *total nitrogen* in urine is also of great importance, and is now usually carried out by Kjeldahl's method.[1] This consists in converting all the nitrogen of a measured portion of urine into ammonia by boiling with fuming sulphuric acid and the subsequent addition of potassium permanganate. The ammonia is then expelled from the acid solution by distillation with an excess of caustic soda or potash, the ammonia being received into a measured volume of standardised acid, whose diminution of acidity due to the absorption of ammonia is finally determined by titration with standard alkali.

The synthesis of urea by molecular transformation of ammonium cyanate indicates an undoubtedly close relationship of urea to cyanic acid, and there are other reactions which enforce the same idea. Thus by the union of water with cyanamide, which is readily affected by treatment with 50 p.c. sulphuric acid, urea is obtained: — $CN.NH_2 + H_2O = (NH_2)_2CO$. It is further stated that when potassium cyanate and acid potassium tartrate are dissolved in water and the mixture is kept for some time, a not inconsiderable amount of urea is formed along with some carbonic acid,[2] thus affording experimental support of Salkowski's view[3] that urea might arise in the body from the union of two molecules of cyanic acid and one of water: $CO.NH+CO.NH+H_2O=(NH_2)_2CO+CO_2$. The final formation of cyanuric acid $(CO.NH)_3$ by the action of heat on dry urea is further evidence in the same direction. On the other hand there are a number of reactions resulting in the production of urea, which leave but little doubt that urea, while closely related to cyanic acid, is truly the amide of carbonic or carbamic acid. Thus by the action of ammonia on phosgene gas:—$COCl_2+2NH_3=CO(NH_2)_2+2HCl$: of ammonia on diethyl-carbonate:—$CO.(C_2H_5O)_2+2NH_3=CO(NH_2)_2+2C_2H_5.OH$:—reactions which are strictly analogous to the formation of acetamide $CH_3.CO(NH_2)$ by the action of ammonia on acetyl chloride $CH_3.COCl$, and on ethyl-acetate $CH_3.COO(C_2H_5)$.

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[1] *Zt. f. anal. Chem.* Bd. XXII. (1883), S. 366.

[2] Hoppe-Seyler, *Physiol. Chemie*, S. 809.

[3] *Zt. f. physiol. Chem.* Bd. I. (1877), S. 41.

It is interesting to observe here that acetamide yields methylcyanide by treatment with phosphorous pentoxide:—$CH_3.CO(NH_2)=CH_3.CN+H_2O$.

Acetamide is also formed by the dry distillation of ammonium acetate, the change being one of simple dehydration; and this reaction is one of general applicability, amides being formed by the removal of one molecule of water from the ammonium salt of a monobasic acid or of two molecules of water from that of a dibasic acid, e.g. ammonium oxalate yields oxamide. Now although urea has not been formed by the dehydration of ammonium carbonate, it is readily rehydrated into the carbonate by the action of acids, alkalis, superheated water, or the urea ferment. Further, if instead of operating on ammonium carbonate the ammonium salt of carbamic acid (see p. 151) be heated in sealed tubes to 140°, or if it be electrolysed with a rapidly commutated current, it loses a molecule of water and is converted into urea.

When the purely chemical facts above stated are applied to the formation of urea in the animal body it is at once obvious that urea might originate from some cyanic source, or from a simple dehydration of ammonium carbonate or carbamate. A full discussion of the possibilities thus indicated lies outside the scope of this work, but it may not be out of place to indicate, as briefly as may be, the various views which have been put forward concerning the probable way in which urea originates in the body.[1]

There is little reason for doubting that the larger part of the nitrogen which leaves the body as urea was at one time a constituent of the nitrogenous muscle-substance (see § 484.) There is equally no doubt, both from general considerations and from the fact that no urea can ever be detected in muscles normally, that the nitrogen does not make its exit from the muscles as ready-made urea. Neither until recently had urea been obtained by any purely chemical means from the products of the decomposition of proteids.

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[1] The literature of the subject is very fully quoted in Bunge's *Physiol. and pathol. Chemistry*, 1890. Lecture XVI. pp. 310–348.

The older statements of Béchamp and Ritter that urea may be obtained from proteids by the action of potassium permanganate have been shown to be erroneous.[1] It is at most possible that a trace of guanidin may be formed, and guanadin can by the action of water be converted into urea and ammonia: $NH.C(NH_2)_2+H_2O=(NH_2)_2CO+NH_3$.[2] Drechsel has however obtained from among the products of the decomposition of casein with concentrated boiling hydrochloric acid and chloride of zinc a base to which he has given the name of 'lysatin.' When boiled with baryta water in excess it yields urea.[3]

What knowledge have we of the possible or probable form under which the nitrogen may make its primary exit from the muscles? The connection of muscle-kreatin with urea-formation has been already discussed (§ 484, 485) and the evidence of the connection may be briefly summed up as follows. A considerable amount of kreatin exists (?) in the muscles at any one time, hence probably a considerable amount is continuously being formed; there is no evidence that any of this kreatin leaves the body as such, hence it is presumably converted into some other substance before being discharged, and this other substance is probably urea, seeing that kreatin may be readily decomposed into urea and sarkosin. There are further reasons for supposing that the nitrogen leaves the muscles as a compound containing comparatively little carbon, and kreatin answers to this requirement, since it contains only four atoms of carbon to three of nitrogen.[4] If this latter view be correct it implies that the nitrogen is not split off in the form of amido-acids, since there is not sufficient carbon in proteids to convert their nitrogen into the amido-acids with which we have to deal in the body. On the other hand when these amido-acids (glycin, leucin, aspartic acid and asparagin) are introduced into the body they are partly converted into urea, so that if formed they would account for a portion at least of the urea excreted.

When proteids are decomposed by caustic alkalis, more especially baryta, or during putrefaction, they yield much ammonium carbonate, which by simple dehydration would give urea. Now although ammonium carbonate, like many other salts of this base, is readily converted into urea when administered to man or other animals, there is no evidence, although it is a possibility, that the nitrogen leaves the tissues as ammonium carbonate.

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[1] Loew, *Jn. f. prakt. Chem.* (2) Bd. II. (1870), S. 289. Tappeiner, *Kön. sächs. Gesell. d. Wiss.* 1871. See Abst. in Maly's *Bericht.* 1871, S. 11.

[2] Lossen, *Ann. d. Chem. u. Pharm.* Bd. CCI. (1880), S. 369.

[3] *Ber. d. d. chem. Gesell.* 1890, S. 3096. Cf. *Arch. f. Physiol.* Jahrg. 1891, S. 254 et seq.

[4] Bunge, *loc. cit.* pp. 320, 328.

The above statements seem to embrace all that can be suggested as to the tissue-antecedents of urea, and it remains now to consider the probable mode and seat of their conversion into urea. As regards kreatin it may be that it is split up into urea and sarkosin, the latter being, like other amido-acids, also converted into urea. When the amido-acids are compared with urea it is not conceivable, with our present chemical knowledge, how they can give rise to urea in any way other than by being broken down into an ammonia stage and a subsequent synthesis of urea from this product. The synthesis may however involve any one of the three following processes. The ammonia may unite with carbonic acid to form ammonium carbonate, which is then dehydrated into urea (Schmiedeberg). Again, it may unite with carbamic acid to form the carbamate, which again by loss of one molecule of water yields urea (Drechsel).[1] But in the third place the ammonia residues may unite with some cyanic compound to form urea in accordance with the possibilities indicated above (pp. 156, 160) (Salkowski and Hoppe-Seyler). The view that some cyanic residues may be involved in the formation of urea, while at present devoid of any striking positive evidence in its support, is at first sight most attractive, especially when it is borne in mind how great the molecular energy of the cyanogen compounds is, so that during their degradation in the tissues much energy would be set free. Pflüger,[2] following Liebig, has called attention to this great molecular energy of the cyanogen compounds, and has suggested that the functional metabolism of protoplasm, by which energy is set free, may be compared to the conversion of the energetic unstable cyanogen compounds into the less energetic and more stable amides. In other words, ammonium cyanate is a type of living, and urea of dead nitrogen, and the conversion of the former into the latter is an image of the essential change which takes place when a living proteid dies.

If we accept this view it is perhaps difficult to understand how the cyanic compounds, poisonous as they are known to be, could play a part in the body. But it is apparently the (CN) group which confers on the compounds their poisonous properties; and if cyanic acid be truly carbamide CO . NH this group is non-existent in it, and it has been recently stated that cyanuric acid $(CO . NH)_3$ when introduced into the body leads to an increased excretion of urea.[3]

One difficulty in connection with this view is that as yet cyanic acid has never been obtained by the artificial decomposition of proteids. But on the other hand the proteids are the chief and only source of the cyanogen compounds, for which the starting-point is found in ferrocyanide of potassium, prepared by fusing nitrogenous animal refuse with potassium carbonate and iron. There is further evidence of the existence in the body of cyanic residues, as shown by the exit from it of sulphocyanates (HCNS), which are found in both saliva and more particularly in urine.[1] The existence of sulphur in these salts suggests at once that it arises from the decomposition of proteids, into whose composition sulphur enters as a constant and characteristic constituent. The formation of sulphocyanic acid in the body has recently been investigated, and it is worthy of note that it is stated to occur in the urine only of those animals which excrete their nitrogen chiefly in the form of urea.[2]

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[1] Cf. above sub sarkosin, p. 140, and carbamic acid, p. 151.

[2] Pflüger's *Arch.* Bd. x. (1875), S. 337.

[3] Coppola, *Rendic. d. R. Acc. d. Lincei,* 1889, pp. 378, 668. *Ann. di Chim. e di farmac.* (4) T. x. (1889), p. 3.

The various ways by which it has been suggested that urea may arise in the body all imply that whatever be the form in which the nitrogen initially leaves the tissues, the substance or substances in which it makes its exit undergo their final (synthetic ?) conversion in some other organ of the body. In the case of leucin there is distinct evidence that the conversion is effected in the liver, and there is increasing evidence that this organ is largely concerned in the presumably synthetic changes which lead to the formation of urea in mammals and of uric acid in birds. Thus Schröder has shown that the conversion of ammonium carbonate into urea occurs in the liver,[3] and a similar relationship to the formation of uric acid in birds has additionally been proved.[4] Further there are many observations which show, when the liver is diseased, a marked diminution in the excretion of urea, with a frequently increased output of ammonia.[5] After extirpation of the liver in birds the urine contains not only more ammonia but a large amount of sarcolactic acid.[6] It would be however premature to regard this fact as showing that in birds uric acid is partly formed by the converting activity of the liver brought to bear upon ammonia and lactic acid. When urea is given to birds it reappears externally as uric acid,[7] but this change is not effected after extirpation of the liver.

Substituted Ureas. The hydrogen atoms of urea can be replaced by alcohol- and acid-radicles. The results are substituted ureas in the first case, or ureides as they are called in the second, when the hydrogen is replaced by the radicle of an acid. Many of them are called acids, since the hydrogen from the amido group, if not all replaced as above, can be replaced by a metal. Thus the substitution of oxalyl

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[1] Munk, Virchow's *Arch.* Bd. LXIX. (1877), S. 354. Gscheidlen, Pflüger's *Arch.* Bd. XIV. (1877), S. 401.

[2] Bruylants, *Bull. de l'acad. de méd. de Belgique*, (4) T. II. (1888), p. 18 *et seq.*

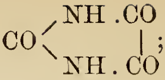
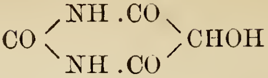
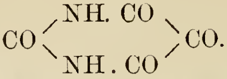
[3] *Arch. f. exp. Path. u. Pharm.* Bd. XV. (1882), S. 364; Bd. XIX. (1885), S. 373. Cf. W. Salomon, Virchow's *Arch.* Bd. XCVII. (1884), S. 149.

[4] Minkowski, *Arch. f. exp. Path. u. Pharm.* Bd. XXI. (1886), S. 40.

[5] Roster, *Lo Sperimentale*, T. XLIV. (1879), p. 153. Hallervorden, *Arch. f. exp. Path. u. Pharm.* Bd. XII. (1880), S. 237. Stadelmann, *Deutsch. Arch. f. klin. Med.* Bd. XXXIII. (1883), S. 526.

[6] Minkowski, *loc. cit.* See also Marcuse, Pflüger's *Arch.* Bd. XXXIX. (1886), S. 425.

[7] Meyer u. Jaffé, *Ber. d. d. chem. Gesell.* Bd. X. (1877), S. 1930.

(oxalic acid) gives parabanic acid,  $CO\left\langle\begin{array}{l}NH.CO\\ \quad\;\;|\\ NH.CO\end{array}\right.$; of tartronyl (tartronic acid), dialuric acid,  $CO\left\langle\begin{array}{l}NH.CO\\ NH.CO\end{array}\right\rangle CHOH$; of mesoxalyl (mesoxalic acid), alloxan  $CO\left\langle\begin{array}{l}NH.CO\\ NH.CO\end{array}\right\rangle CO$. These substances are interesting as being also obtained by the artificial oxidation of uric acid. The close chemical relationship of urea to uric acid will be explained below.

Uric acid. $C_5H_4N_4O_3$.

The chief constituent of the urine in birds and reptiles; it occurs only sparingly in this excretion in man (·2 – 1 grm. in 24 hours) and most mammalia. It is normally present in the spleen, and traces of it have been found in the lungs, muscles of the heart, pancreas, brain, and liver. Urinary and renal calculi often consist largely of this substance, or its salts. In gout, accumulations of uric acid salts may occur in various parts of the body, more especially at the joints, forming the so-called gouty concretions.

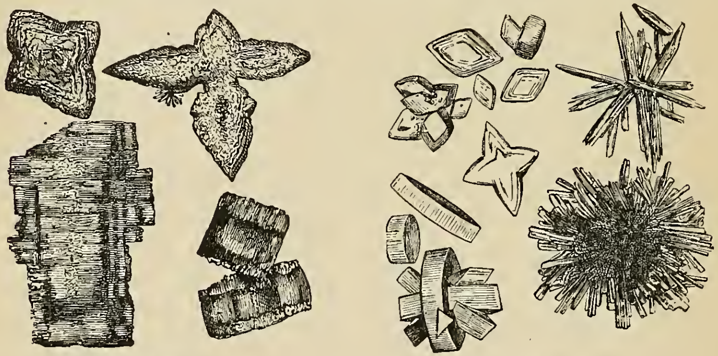

Rapidly separated. Slowly separated.

Fig. 17. Crystals of Uric Acid. (Krukenberg after Kühne.)

It is when pure a colourless, crystalline powder, tasteless, and without odour. The crystalline form is very variable, differing according to the concentration of the solution from which the crystals are obtained, the rate at which they are formed, and whether they are separated out spontaneously or by the addition of acids to either solutions of the acid or to urine. Hence it is extremely difficult to illustrate them within reasonable limits, and for figures of the various possible forms some special work must be consulted.[1] The impure acid crystallises much more readily than does the purified. The following figure shows additionally some very characteristic forms in which uric acid separates out from urine either spontaneously or after the condition of hydrochloric acid.

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[1] See Ultzmann and K. B. Hofmann, *Atlas der Harnsedimente*, Wien, 1872. Also Funke, *Atlas d. physiol. Chem.* Leipzig, 1858.

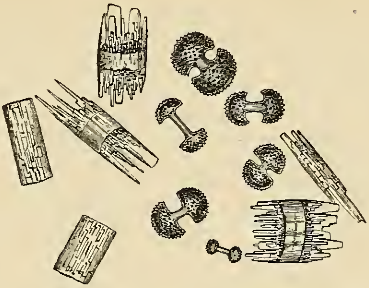

FIG. 18. CRYSTALS OF URIC ACID. (After Funke.)

Uric acid is remarkably insoluble in water (1 in 14,000 or 15,000 of cold water, 1600 of boiling). Ether and alcohol do not dissolve it appreciably. On the other hand, sulphuric acid takes it up in the cold without decomposition, and it is also readily soluble in many salts of the alkalis, as in the caustic alkalis themselves; ammonia however scarcely dissolves it, and in this respect it differs conveniently from cystin. It is fairly soluble in glycerin, and soluble to some extent in solutions of lithium carbonate.

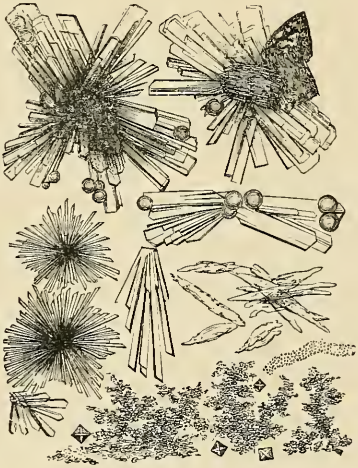

FIG. 19. (Krukenberg after Kühne.)

Urinary sediment, showing chiefly the most usual form of crystals of acid sodium urate, $C_5H_3NaN_4O_3$.

Salts of Uric acid. Of these the most important are the acid urates of sodium, potassium, and ammonium; these salts are frequently still called 'lithates,' the term 'lithic' acid being used for uric acid. The sodium salt which is the most common constituent of many urinary sediments crystallises in many different forms, these not being characteristic, since they are almost the same for the corresponding compounds of the other two bases. It is very sparingly soluble in cold water (1 in 1100 or 1200), more soluble in hot (1 in 125). It is the principal constituent of several forms of urinary sediment, and composes a large part of many calculi; the excrement of snakes contains it largely. The potassium resembles the sodium salt very closely, as also does the compound with ammonium; the latter occurs generally in the sediment from alkaline urine.

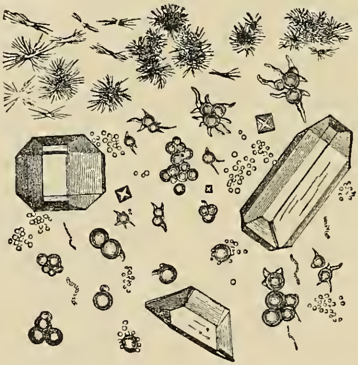

Fig. 20. (Krukenberg after Kühne.)

Urinary sediment from alkaline urine. The large crystals consist of ammonio-magnesium phosphate (triple phosphate, $NH_4MgPO_4 + 6H_2O$). A few crystals (octahedra) of calcium oxalate are also shown. The remaining crystals represent the form of acid ammonium urate, $C_5H_3(NH_4)N_4O_3$. The rounded objects are urinary fungi.

Preparation. The amount of uric acid in mammalian urine is too small to make it a source of the acid. Crystals may however be readily obtained from human urine by adding to it 2 — 3 p.c. of strong hydrochloric acid and letting it stand for one or two days in a cool place. The crystals form on the sides of the containing vessel.

On the large scale it is usually prepared from guano, or from snake's excrement. From the latter it is obtained by boiling with caustic potash (1 part alkali to 20 of water) as long as ammonia is evolved; in the filtrate a precipitate of acid urate of potassium is formed by passing a current of carbonic acid; this salt is then washed, dissolved in caustic potash, and decomposed by carefully filtering its solution into an excess of dilute hydrochloric acid.

By similar treatment uric acid is readily obtained from fowl's excrement, a convenient source of the acid.

Identification of uric acid. The crystalline forms afford some clue, but are so numerous that some forms which may at any time present themselves are scarcely characteristic. The rhombic tables, 'dumb-bell,' and 'whetstone' crystals are on the whole most characteristic.

i. *Murexid test.* The suspected substance is treated in a porcelain dish with a few drops of strong nitric acid and evaporated *carefully* to dryness, by preference on a water-bath. The residue thus obtained will, if uric acid is present, be of a yellow or more frequently red colour, which turns to a brilliant reddish purple on exposure to the vapours of ammonia. On the subsequent addition of a drop of caustic soda the colour is changed to a reddish blue. This disappears on warming, whereas the similar colour obtained by the above process from guanin does not. This is an important means of distinguishing between the two substances.

The test depends on the formation of murexid, which is the acid ammonium salt of purpuric acid, the acid itself being unknown in the free state. Uric acid is decomposed when heated with nitric acid, yielding alloxan and then alloxantin ; by the action of ammonia the latter is converted into murexid $(NH_4)\ C_8H_4N_5O_6 + H_2O$.

The murexid test is so striking and characteristic that it suffices completely for the identification of uric acid. The following tests may be applied in confirmation if required, but not for the purposes of initial detection.

ii. *Schiff's reaction.*[1] The substance is dissolved in sodium carbonate, and a drop is then placed on filter paper previously moistened with nitrate of silver. A yellow or almost black colouration, due to the formation of metallic silver by reduction of its nitrate, is at once obtained.

iii. When a solution of uric acid in caustic soda is boiled with a small amount of Fehling's fluid, reduction occurs with production of a greyish precipitate of urate of cuprous oxide. If the copper salt is in excess red cuprous acid is obtained.

Estimation of uric acid in solutions (urine). The accurate quantitative determination of uric acid is a matter of some difficulty; for details some standard works (quoted sub urea) should be consulted. It will suffice to indicate here the principles of the more usually employed methods.

i. *Salkowski-Ludwig method.*[2] When an ammoniacal solution of nitrate of silver is added to a solution of uric acid, to which an ammoniacal mixture of magnesium chloride and ammonium chloride has been previously added, the uric acid is precipitated as a magnesio-silver salt. This is collected, washed, and decomposed by sodium or potassium hydrosulphide, whereupon the uric acid passes again into solution as a urate of the alkali. On the addition of an excess of hydrochloric acid to this solution the urate is decomposed, uric acid separates out and is collected and weighed.

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[1] *Ann. d. Chem. u. Pharm.* Bd. CIX. (1859), S. 65.

[2] Ludwig, *Wien. med. Jahrb.* 1884, S. 597. Cf. Camerer, *Zt. f. Biol.* Bd. XXVII. (1890), S. 153.

ii. *Haycraft's method.*[1] When uric acid is precipitated by ammoniacal solution of nitrate of silver in presence of the ammonio-magnesic mixture as above described the precipitate is stated to contain one atom of silver to each molecule of uric acid. The uric acid is hence determined by dissolving the precipitate in nitric acid, in which solution the silver is then estimated volumetrically with a standard solution of potassium sulphocyanate.[2]

Chemical constitution of uric acid. Notwithstanding the frequent and careful investigation of uric acid and of the extremely numerous products of its decomposition, its constitution has until recently been a matter chiefly of surmise and conjecture, and many constitutional formulæ have been assigned to it. When uric acid is treated with concentrated hydriodic acid at 160–170° it is decomposed into glycin, ammonia, and carbonic anhydride

$$C_5H_4N_4O_3 + 5H_2O = CH_2(NH_2) . COOH . + 3CO_2 + 3NH_3.$$

By reversing this decomposition as it were, namely by fusing together at 200 – 230° glycin and urea, uric acid was for the first time obtained artificially;[3] when sarkosin is used instead of urea methyl-uric acid is obtained. Uric acid has also been prepared by fusing together trichlor-lactamide or trichlor-acetic acid and urea.[4] The high temperatures at which the above reactions were conducted and the uncertainty as to the nature of the products intermediate between the reagents and the finally formed uric acid precluded them from being regarded as syntheses in the strict sense of the word. A true synthesis of uric acid has been recently discovered by Behrend and Roosen,[5] from which it appears that the constitutional formula first assigned to the acid by Medicus,[6] is a true representation of its constitution. This view had been previously stated by E. Fischer as a result of his analytical investigations of uric acid.[7]

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[1] *Brit. Med. Jl.* 1885, p. 1100. *Jl. of Anat. and Physiol.* Vol. xx. p 695. *Zt. f. anal. Chem.* Bd. xxv. (1885), S. 165. *Zt. f. physiol. Chem.* Bd. xv. (1891), S. 436.

[2] Volhard, *Jn. f. pr. Chem.* (2) Bd. ix. (1874), S. 217.

[3] Horbaczewski, *Monatsh. f. Chem.* Bd. iii. (1882), S. 796. *Ber. d. deutsch. chem. Gesell.* Jahrg. (1882), S. 2678.

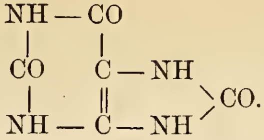
[4] Horbaczewski, *Monatsh. f. Chem.* Bd. vi. (1885), S. 356; Bd. viii. (1887), Sn. 201, 584.

[5] *Ann. d. Chem. u. Pharm.* Bd. ccli. (1889), S. 235.

[6] *Ibid.* Bd. clxxv. (1875), S. 230.

[7] *Ber. d. deutsch. chem. Gesell.* 1884, Sn. 328, 1785.

Uric acid.

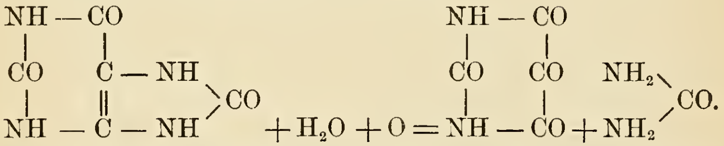


An inspection of the above formula shows at once that uric acid contains the residues of two molecules of urea. This corresponds to the fact that nearly all the possible decompositions of uric acid yield either a molecule of urea along with the more specific product of the decomposition, frequently itself a derivative of urea, or else some substance which can by further change be decomposed into urea and some other product which is as before frequently a derivative of urea. The close chemical relationship of urea and uric acid is thus clearly shown, and may be further emphasized by the following reactions, which illustrate and amplify at the same time the general statement which has just been made.

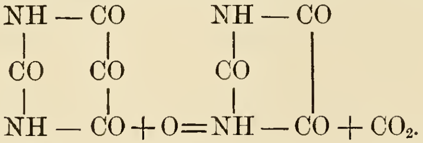
The decomposition of uric acid takes place in two stages, yielding two series of products, of which one is headed by alloxan and the other by allantoin; from these two substances respectively the other members of each series are derived by subsequent decomposition.

1. *Alloxan series.*

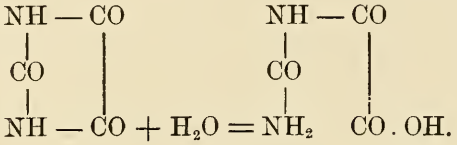
By careful oxidation with nitric acid uric acid is decomposed into a molecule of alloxan and one of urea.



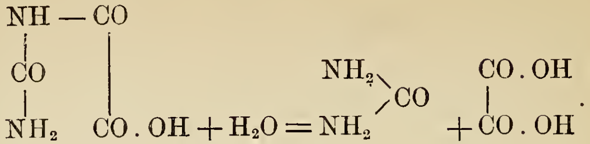
Alloxan is itself a substituted urea or ureide (antea, p. 164), viz. mesoxalyl-urea, and by oxidation can be further converted into parabanic acid (oxalyl-urea) and carbonic anhydride.



By heating with alkalis parabanic acid is hydrated and yields oxaluric acid.

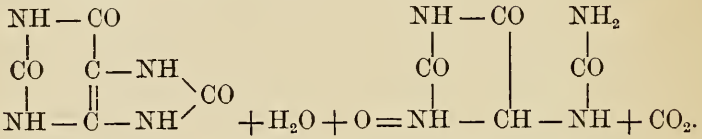


The latter by prolonged boiling with water is converted into urea and oxalic acid.

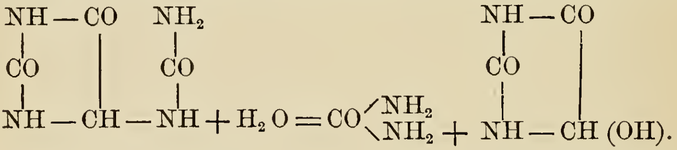


2. *Allantoin series.*

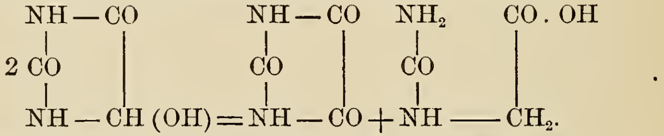
By oxidation with potassium permanganate uric acid is decomposed into allantoin and carbonic anhydride.



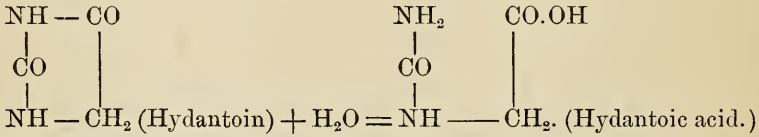
When allantoin is boiled with nitric acid it is hydrated and decomposes into a molecule of urea and one of allanturic acid.



Allanturic acid is itself a substituted urea, viz. glyoxyl-urea, and may be converted into parabanic and hydantoic acids.

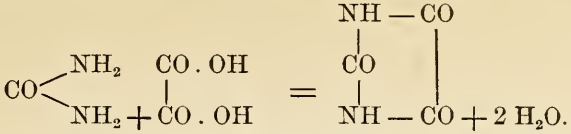


Of these two acids the parabanic may as before be converted into oxalic acid and urea, and hydantoic acid is a derivative, by simple hydration, of hydantoin, which is itself a substituted urea, viz. glycolyl-urea, containing a residue of glycolic acid, [$CH_2(OH).COOH$].



The above reactions and decompositions show clearly how close is the chemical relationship of urea and uric acid, and the connection is still more evident when it can be shown that many of the products described above as obtained during the decomposition of uric acid, viz. the ureides, can be prepared from urea directly.

Thus parabanic acid (oxalyl-urea) is readily formed by the action of phosphorus oxychloride on a mixture of urea and oxalic acid:



$$CO\!\begin{matrix}\diagup NH_2\\ \diagdown NH_2\end{matrix} + \begin{matrix}CO.OH\\ |\\ CO.OH\end{matrix} = \begin{matrix}NH-CO\\ |\qquad |\\ CO\qquad |\\ |\qquad |\\ NH-CO\end{matrix} + 2\,H_2O.$$

When the close chemical relationship of urea to uric acid is taken into account, the statement that those substances which when introduced into the body of a mammal lead to an increased excretion of urea, when introduced into the organism of birds are converted into uric acid,[1] needs excite no surprise. There is further distinct evidence, already referred to under urea, that the conversion is affected in the liver.[2] We know nothing as yet as to the cause of the slight divergence of metabolism which leads to the preponderating formation of urea in mammals and of uric acid in birds and reptiles. It is certainly not due, as some have supposed, to insufficient oxidation in the latter, since the excretion of uric acid is not increased in mammals by artificial disturbance of the respiratory interchange,[3] and it is exactly in birds that the most active oxidational changes, as shown by their higher temperature, is observed. Bearing in mind how readily uric acid yields urea as one product of its oxidational decomposition, it has been supposed that a good deal more uric acid is formed in the mammalian body than is excreted in the urine. In support of this view it may be pointed out that uric acid when introduced into mammals is largely excreted as urea, and that some of the known products of the artificial oxidation of uric acid are occasionally found in their urine, e.g. oxalic acid, oxaluric acid (hydrated parabanic acid), and allantoin.[4] The latter substance is apparently increased (?) by the administration of uric acid.[5]

3. **Oxaluric acid.** $C_3H_4N_2O_4$. (Hydrated parabanic acid.)

Occurs in minute traces in normal urine, from which it is extracted by filtering a large quantity of urine very slowly through a relatively small amount of animal charcoal. The charcoal after being washed with distilled water is extracted with boiling alcohol, to which it yields the oxaluric acid as an ammonium salt. The free acid is a white crystalline powder, not very soluble in water: its alkaline salts are readily soluble.[6]

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[1] For literature see Bunge, *Physiol. path. Chemistry*, p. 341. Horbaczewski, *Monatshft. f. Chem.* Bd. x. (1889), S. 624. *Sitzb. d. Wien. Akad.* Bd. xcviii. (1889), 3 Abth. S. 301.

[2] See also von Schröder, *Arch. f. Physiol.* 1880. Suppl.-Bd. S. 113. Ludwig's *Festschrift*, 1887, S. 98.

[3] Senator, Virchow's *Arch.* Bd. xlii. (1868), S. 35.

[4] Salkowski u. Leube, *Die Lehre vom Harn* (1882), S. 100.

[5] Salkowski, *Ber. d. d. chem. Gesell.* 1876, S. 719, 1878, S. 500.

[6] For details see Hoppe-Seyler, *Phys.-path. Anal.* 1832, S. 159. Neubauer u. Vogel, *Harnanalyse*, 1890, S. 239.

4. **Allantoin.** $C_4H_6N_4O_3$. (Diureide of glyoxylic acid.)

The characteristic constituent of the allantoic fluid, more especially of the calf, as also in fœtal urine and amniotic fluid; it occurs also in the urine of many animals for a short period after their birth. Traces of it are sometimes detected in this excretion at a later date. It is obtained in urine after the internal administration of uric acid.[1] It has also been found in vegetable tissues.[2] It crystallises in small, shining, colourless, hexagonal prisms. They are soluble in 160 parts of cold water, more soluble in hot, insoluble in cold alcohol and ether, soluble in hot alcohol. Carbonates of the alkalis dissolve them, and compounds may be formed of allantoin with metals but not with acids. The salts with silver and mercury are important as providing a means of separating allantoin from its solutions.

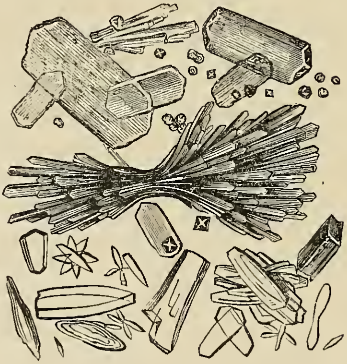

Fig. 21. Crystals from concentrated Urine of Calf. (After Kühne.)

The large central crystal composed of an aggregation of small prisms is allantoin: those below it are crystals of kreatin, kreatinin and oxalate of lime. The large prisms in the upper part of the figure consist of magnesium phosphate.

Allantoin gives no reactions which are sufficiently striking to admit of its detection in urine or other fluids; it must therefore in all cases first be separated out and then examined. The separation may be effected in several ways, of which those more usually employed consist in its precipitation with nitrate of mercury or silver.[3] From the urine of calves or from their allantoic fluid, allantoin may usually be obtained in crystals by mere concentration and subsequent standing till crystallisation occurs.

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[1] Salkowski, *loc. cit.*

[2] Schulze u. Barbieri, *Jn. f. pr. Chem.* Bd. xxv. (1882), S. 145. Schulze u. Bosshard, *Zt. f. physiol. Chem.* Bd. ix. (1885), S. 420.

[3] For details see Hoppe-Seyler, *loc. cit.* S. 162. Neubauer u. Vogel, *loc. cit.* S. 222.

Preparation. Allantoin may be easily obtained by the careful oxidation of uric acid with potassium permanganate.[1] It may also be synthetised by prolonged heating to 100° of a mixture of glyoxylic acid and urea,[2] or of the latter substance with mesoxalic acid.[3]

As prepared artificially it crystallises readily in large prismatic hexagonal crystals.

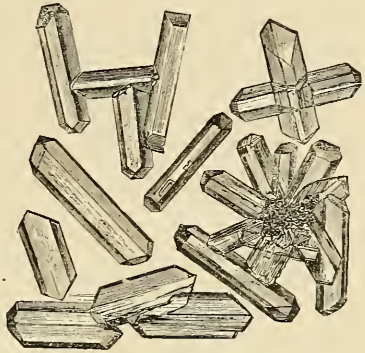

FIG. 22. CRYSTALS OF ALLANTOIN PREPARED BY THE OXIDATION OF URIC ACID. (After Kühne.)

In addition to the crystalline form and precipitability with nitrates of mercury and silver, allantoin is further characterised by yielding Schiff's reaction with furfurol (see above, p. 158, sub urea), but less readily and with less intense colouration than does urea. It also reduces Fehling's fluid on prolonged boiling.

THE XANTHIN GROUP.[4]

This group comprises a number of substances closely related to uric acid and to each other. Some of them occur in small amounts in the tissues (muscles) and excretions (urine) of the body and are to be regarded as being, like urea and uric acid, typical products of the downward destructive metabolism of proteids. Some of them are closely related to certain alkaloids which occur in plants (theobromin and caffeïn), and which probably play some not unimportant part in the nutritional changes of the animal body, since they are constantly consumed, in some form or other, by the larger part of the human race. This relationship of the xanthin-bodies to certain vegetable alkaloids is further interesting when it is

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[1] Claus, *Ber. d. d. chem. Gesell.* Bd. VII. 1874, S. 227.

[2] Grimaux, *Compt. Rend.* T. 83 (1876), p. 62.

[3] Michael, *Amer. Chem. Jl.* Vol. V. (1883), p. 198.

[4] For a full statement of the general reactions of this group, and the methods for their separation and discrimination, see Neubauer u. Vogel, *Analyse des Harns,* 1890. Sec. 200—219.

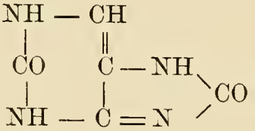
The following tabular statement shows how close is the relationship of the members of this group to uric acid, to each other, and to certain vegetable alkaloids, and further, by means of guanidin (an oxidation product of guanin) and uric acid to urea, and also by means of guanidin to kreatin (see p. 143).

Uric acid	$C_5H_4N_4O_3$			
Xanthin	$C_5H_4N_4O_2$. .	Heteroxanthin (Methyl-xanthin?).	$C_6H_6N_4O_2$	
		Paraxanthin (Dimethyl-xanthin?).	$C_7H_8N_4O_2$	Theobromin (Dimethyl-xanthin). $C_7H_8N_4O_2$.
				Theophyllin (Isomeride of Theobromin). $C_7H_8N_4O_2$.
				Caffeïn or Theïn (Trimethyl-xanthin). $C_8H_{10}N_4O_2$.
		Carnin.	$C_7H_8N_4O_3$	
Hypoxanthin (or Sarkin)	$C_5H_4N_4O$			
Adenin	$C_5H_5N_5$	(Bears the same relationship to hypoxanthin that guanin does to xanthin).		
Guanin	$C_5H_5N_5O$	(Guanidin. CN_3H_5 Product of the oxidation of guanin with simultaneous formation of parabanic acid: — oxalyl-urea).		

remembered that the latter are regarded by plant-physiologists as waste-products of the vegetable organism, and are thus found chiefly in those parts of the plant which are on their way to removal, viz. the bark, leaves, and seeds.

Many members of this group are both derivable from and convertible into other members of the group by simple chemical processes, but this relationship of the one to the other will be more fully appreciated by consideration of the properties and reactions of the separate substances. Their relationships to uric acid and each other are in many cases indicated by comparison of their formulae.

1. **Xanthin.** $C_5H_4N_4O_2$.



(Fischer)[1].

First discovered in a urinary calculus, and called xanthic oxide. More recently it has been found as a normal, though very scanty, constituent of urine, muscles, and several other tissues, such as the liver, spleen, thymus, brain-substance, &c. It occurs in larger quantities, together with hypoxanthin, in 'extract of meat,' and is also found in traces in vegetable tissues, — lupins, malt-seedlings, and tea. In nearly all cases it is accompanied by hypoxanthin. The amount which is present in any of the above tissues and fluids is so small that none of them, except perhaps the extract of meat, affords a convenient source for its preparation.[2] To obtain it in quantity guanin is treated with nitrous acid,[3] and the nitro-product thus obtained is reduced in ammoniacal solution with ferrous sulphate. It may also be prepared artificially from hydrocyanic acid and water in presence of acetic acid.[4] When pure it is a colourless powder, requiring about 14,000 parts of water for its solution at ordinary temperatures,

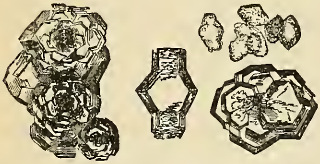


FIG. 23. XANTHIN HYDROCHLORIDE, $C_5H_4N_4O_2 . HCl$. (Kühne.)

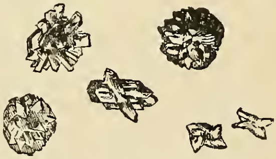


FIG. 24. XANTHIN NITRATE, $C_5H_4N_4O_2 . HNO_3$. (Kühne.)

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[1] (i) *Ber. d. d. chem. Gesell.* 1882, S. 453. (ii) *Ann. d. Chem. u. Pharm.* Bd. CCXV. (1882), S. 253.

[2] For its separation from urine see Neubauer, *Zt. f. anal. Chem.* Bd. VII. (1868), S. 398. From muscle-extract, see Städeler, *Ann. d. Chem. u. Pharm.* Bd. CXVI. (1860), S. 102. Neubauer, *Zt. f. anal. Chem.* Bde. II. (1863), S. 26, VI. (1867), S. 33.

[3] Fischer, *loc. cit.* (ii).

[4] Gautier, *Compt. Rend.* T. 98 (1884), 1523.

and 1400 at 100°. Insoluble in alcohol and in ether, it dissolves readily in dilute acids and alkalis (characteristically in ammonia) forming crystallisable compounds.

Reactions. The discrimination of members of the xanthin group is not easy, since from their close relationship they yield many reactions in common. The following are characteristic of xanthin.

i. *Weidel's reaction.*[1] The substance is warmed with freshly prepared chlorine-water and a trace of nitric acid as long as any gas is evolved: it is then carefully evaporated to dryness and, if xanthin is present, the residue turns pink or purplish-red on the access of ammonia fumes. Carnin gives a similar colouration if but little chlorine-water is used, while guanin and adenin do not.

ii. *Hoppe-Seyler's reaction.* When xanthin is introduced into some caustic soda with which some chloride of lime has been mixed, each particle of the substance surrounds itself with a dark green ring which speedily turns brown and then disappears.

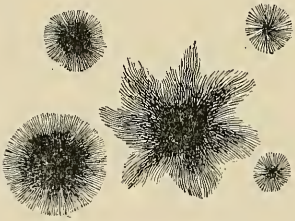

Fig. 25. Crystals of Xanthin silver-nitrate, $C_5H_4N_4O_2 . AgNO_3$. (Krukenberg after Kühne.)

iii. *Strecker's test.*[2] When evaporated to dryness on porcelain with nitric acid a yellow residue is obtained which turns reddish-yellow on the addition of caustic soda or potash (not of ammonia), and reddish-violet on subsequent warming. Distinctive from uric acid.

iv. Xanthin is more readily soluble in ammonia than is uric acid.

v. Xanthin yields in solution in dilute nitric acid a characteristic crystalline compound with nitrate of silver, which differs from the similar compound of hypoxanthin both in the forms which it presents and in its greater solubility in nitric acid of sp. gr. 1·1 at 100°. It is therefore used as a means of separating xanthin and hypoxanthin.

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[1] *Ann. d. Chem. u. Pharm.* Bd. CLVIII. (1871), S. 365. This reaction was given by its author for hypoxanthin, but apparently in error. Cf. Kossel, *Zt. f. physiol. Chem.* Bd. VI. (1882), S. 426. Salomon, *Ber. d. d. chem. Gesell.* 1883, S. 198.

[2] *Ann. d. Chem. u. Pharm.* Bd. CVIII. (1858), S. 146.

vi. The compound of xanthin with hydrochloric acid is far less soluble in water than are the similar compounds of hypoxanthin and guanin, and hence affords a further means of separating these bases.

By treatment with hydrochloric acid and potassium chlorate xanthin is converted into alloxan and urea (Fischer).

The older and frequently repeated statements that xanthin and hypoxanthin can be obtained from uric acid by the action of sodium-amalgam, as also that hypoxanthin can be converted into xanthin by treatment with nitric acid, have recently been shown to be erroneous. Notwithstanding the similarity of their composition these three substances are incapable of interconversion.[1]

2. **Heteroxanthin.** $C_6H_6N_4O_2$ (Methyl-xanthin?).

This substance occurs in minute quantities in the normal urine of man[2] and the dog,[3] along with xanthin and hypoxanthin and another closely allied xanthin-base, paraxanthin. It occurs in larger amount in the urine of leukhaemic patients. It is crystalline, but not very characteristically so, is soluble with difficulty in cold water, much more soluble in hot water, is insoluble in alcohol and in ether. It may, as also may paraxanthin, be separated from other xanthin-bases by taking advantage of the relatively slight solubility of its sodium salt in caustic soda. It also yields with hydrochloric acid a relatively insoluble salt which crystallises readily, whereas the corresponding salt of paraxanthin is readily soluble. They may by this means be separated the one from the other.

Heteroxanthin does not give the ordinary reaction for xanthin with nitric acid and caustic soda, but yields a brilliant colouration on the application of Weidel's test (see sub xanthin). Like the other xanthin-bases it gives an insoluble salt with an ammoniacal solution of nitrate of silver.

3. **Paraxanthin.** $C_7H_8N_4O_2$ (Dimethylxanthin ?) Isomeride of Theobromin.

Like heteroxanthin it occurs in very small amounts in urine.[4] It is soluble with difficulty in cold water, but is more soluble than xanthin; is much more soluble in hot water, insoluble in alcohol and in ether. It crystallises readily in characteristic flat, somewhat irregular, six-sided tables when its solutions are slowly evaporated, or in needles if rapidly. It forms, as do the preceding substances, a crystalline salt with nitrate of silver; this like the corresponding compound of xanthin is soluble in strong nitric acid (sp. gr. 1·1) at 100°, and may thus be separated from hypoxanthin. It may be separated from xanthin by means of its greater solubility in cold water, and from heteroxanthin by the difference in the solubility of its salts with sodium and hydrochloric acid.

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[1] Kossel, *Zt. f. physiol. Chem.* Bd. VI. (1882), S. 428. Fischer, *Ber. d. d. chem. Gesell.* 1884, S. 328.

[2] Salomon, *Ibid.* 1885, S. 3407.

[3] Salomon, *Zt. f. physiol. Chem.* Bd. XI. (1887), S. 412.

[4] Thudichum, *Annals of ch. Med.* Vol I. (1879), p. 166. Salomon, *Ber. d. d. chem. Gesell.* 1883, S. 195, 1885, 3406, *Zt. f. klin. Med.* Bd. VII. (Suppl.-Hft.) (1884), S. 63. Cf. *Zt. f. physiol. Chem.* Bd. XV. (1891), S. 319.

Paraxanthin gives Weidel's reaction but not the ordinary xanthin test with nitric acid and caustic soda.

An inspection of Fischer's formula for xanthin shows the possibility of the existence of at least two isomeric di-methyl derivatives of this base according to the replacement by methyl CH_3 of the hydrogen atoms in the three NH groups which it contains. Of these one has for some time been known as theobromin; paraxanthin is probably another isomer, and more recently Kossel has described a third, theophyllin. By substitution of (CH_3) for hydrogen in the third (NH) group trimethyl-xanthin or caffeïn is obtained. In connection with the isomeric relationship of paraxanthin and theobromin it is of great interest to observe that the physiological action of the two bases is the same.[1]

4. **Carnin.** $C_7H_8N_4O_3$.

Closely allied in composition to the preceding base, but as yet of unknown constitution, carnin occurs only as a constituent of 'extract of meat,' of which it forms about one per cent.,[2] although it has been stated to occur also in urine (?).[3]

It is prepared by precipitating extract of meat with baryta-water, avoiding all excess of the precipitant. The filtrate from this is now precipitated with basic acetate of lead, which carries down all the carnin. This precipitate is repeatedly boiled with water which dissolves out the lead salt of carnin, which is then decomposed by sulphuretted hydrogen, and the carnin obtained by concentration of the aqueous filtrate from the sulphide of lead.[4]

It crystallises in white masses composed of very small irregular crystals; it is soluble with great difficulty in cold, readily soluble in hot water, insoluble in alcohol and in ether. It unites with acids and salts to form crystallisable compounds. Of these the more important are the salts with basic lead acetate, soluble in boiling water, and with nitrate of silver, insoluble in strong nitric acid and ammonia. Carnin gives Weidel's reaction when only a small amount of chlorine-water is employed, but the test fails if any excess is used.

Carnin bears an interesting relationship to hypoxanthin, into which it may be converted by treatment with chlorine or nitric acid, or still more readily by bromine.

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[1] Salomon, *Verh. d. physiol. Gesell.* Berlin. *Arch. f. Physiol.* 1887, S. 582.

[2] Weidel, *Ann. d. Chem. u. Pharm.* Bd. CLVIII. (1871), S. 353.

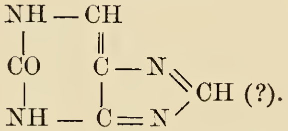
[3] Pouchet, *Journ. de Thérap.* T. VII. (1880), p. 503.

[4] Krukenberg u. Wagner, *Sitzb. d. phys.-med. Gesell.* Würzburg, 1883, No. 4.

$$C_7H_8N_4O_3 + Br_2 = C_5H_4N_4O \,.\, HBr + CH_3Br + CO_2.$$

The latter may be isolated from its hydrobromic acid salt by means of caustic soda.

5. **Hypoxanthin or Sarkin.** $C_5H_4N_4O$.



Closely related to xanthin and usually occurring with it in the tissues and fluids of the body. Its constitutional formula has not yet been definitely ascertained, but it will probably be found to contain the group $N = CH - N$ in the place of one urea residue in xanthin.[1] On this supposition three formulae are obviously possible, and the correct one has still to be determined. Hypoxanthin may be obtained from normal muscles, and hence is found in larger amounts in 'extract of meat.' It occurs also in the spleen, liver, and medulla of bones, and in considerable quantity in the blood[2] and urine[3] of leukhæmic patients; also in normal urine[4] and in vegetable tissues — lupins,[5] malt-seedlings, and tea.[6]

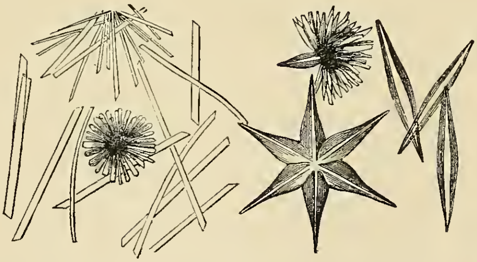


Fig. 26. Hypoxanthin-silver-nitrate, $C_5H_4N_4O \,.\, AgNO_3$. (Krukenberg after Kühne.)

It is obtained from fluids or tissue extracts by means of the processes already mentioned for the extraction of xanthin, and is separated from the latter by taking advantage of the slighter solubility of its salt with nitrate of silver in boiling nitric acid (sp. gr. 1·1). The crystalline form of this salt is characteristic.

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[1] Fischer, *Ber. d. d. chem. Gesell.* 1882, S. 455.
[2] Kossel, *Zt. f. physiol. Chem.* Bd. v. (1881), S. 267.
[3] Stadthagen, Virchow's *Arch.* Bd. cix. (1877), S. 390.
[4] G. Salomon, *Zt. f. physiol. Chem.* Salkowski, Virchow's *Arch.* Bd. l. (1870), S. 195.
[5] Salomon, *Verhand. d. physiol. Gesell.* Nov. 12, 1880. *Arch. f. Physiol.* 1881, S. 166.
[6] Baginsky, *Zt. f. physiol. Chem.* Bd. viii. (1883—4), S. 395.

It also yields crystalline salts with nitric and hydrochloric acids.

Hypoxanthin is soluble in 300 parts of cold and 78 of boiling water, insoluble in cold alcohol and in ether, soluble in 900 parts of boiling alcohol. It does not yield either Weidel's reaction or the reaction with nitric acid and caustic soda so characteristic of the other xanthin bases. It gives no green colouration with caustic soda and chloride of lime such as xanthin does (Hoppe-Seyler's reaction), but after treatment with hydrochloric acid and zinc, it yields a ruby-red colouration on the addition of an excess of caustic soda (Kossel). In this reaction it resembles adenin.

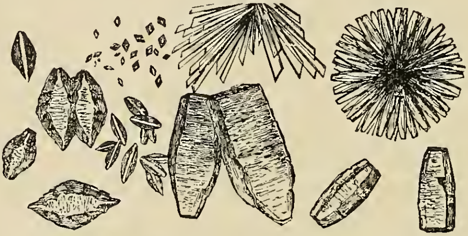

FIG. 27. HYPOXANTHIN-NITRATE, $C_5H_4N_4O \cdot HNO_3$. (Kühne.)

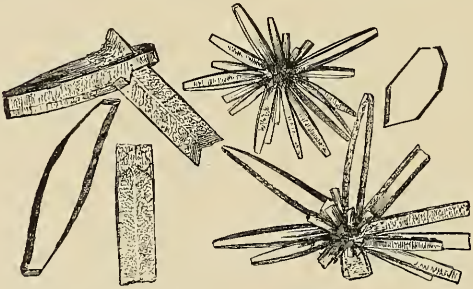

FIG. 28. HYPOXANTHIN-HYDROCHLORIDE, $C_5H_4N_4O \cdot HCl$. (Kühne.)

During the putrefactive decomposition of proteids (fibrin) or by the action of boiling water, dilute acids, or gastric and pancreatic enzymes, hypoxanthin can be obtained in minute amounts.[1] This was at first regarded as evidencing a direct formation of xanthin bases from proteids. The researches of Kossel have however shown that the source of the hypoxanthin in the above cases is probably the nuclein of the corpuscles entangled in the fibrin, since he finds that, by similar treatment, isolated nuclein yields no inconsiderable amount of hypoxanthin.[1] The nuclein however from egg-yolk does not yield hypoxanthin, and thus resembles the nuclein derivable from casein.[2] Although the xanthin-bases undoubtedly result in the body from the metabolism of nitrogenous (proteid) tissues there is as yet no evidence as to the manner in which they can be formed from true proteids.[3] The genetic relationship of hypoxanthin to nuclein probably accounts for the marked occurrence of the former in leukhaemic blood.

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[1] Salomon, *Ber. d. d. chem. Gesell.* 1878, S. 574. Krause, *Inaug.-Diss.* Berlin, 1878. Chittenden, *Jl. of Physiol.* Vol. II. (1879), p. 28.

Bearing in mind the close chemical relationship of uric acid, xanthin, and hypoxanthin, and regarding the xanthin bases as distinctly and typically products of the downward metabolism of nitrogenous tissues, the question at once suggests itself whether in the body there is any antecedental relationship between these substances and uric acid (or urea). As with kreatin (above, p. 162), so with the xanthin bodies, the disproportion between the amount presumably arising in the tissues and that which is actually excreted makes it probable that they are converted into something else, uric acid (or urea), before leaving the body. And in support of this belief there is a certain amount of experimental evidence which was wanting in the case of kreatin. It is found that hypoxanthin administered to a dog does not reappear as such externally in the urine,[4] and that when given to fowls it leads to an increased excretion of uric acid amounting to some 60 p. c. of the hypoxanthin employed.[5] Since the latter result is obtained in fowls with extirpated livers, it appears that the conversion is not effected in this organ, although it is known that normally no inconsiderable portion of the uric acid is formed in their liver.

6. **Adenin.** $C_5H_5N_5$.

This base was obtained by Kossel[6] during the treatment of pancreatic tissue for the preparation of hypoxanthin. It bears the same relationship to the latter that guanin does to xanthin, and can similarly be converted into hypoxanthin by the action of nitrous acid. It is stated to have been found in urine.[7]

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[1] *Zt. f. physiol. Chem.* Bde. III. (1879), S. 284, IV. 290, V. 152, 267, VI. 423, VII. 7. Cf. Löw, Pflüger's *Arch.* Bd. XXII. (1880), S. 62.

[2] Kossel, *Verhandl. d. physiol. Gesell.*, *Arch. f. Physiol.* 1885, S. 346.

[3] Cf. Drechsel, *Ber. d. d. chem. Gesell.* 1880, S. 240. But see also Salomon, *Ibid.* S. 1160.

[4] Baginsky, *Zt. f. physiol. Chem.* Bd. VIII. (1884), S. 397.

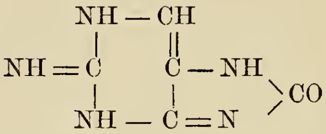
[5] Von Mach, *Arch. f. exp. Path. u. Pharm.* Bde. XXIII. (1887), S. 148, XXIV. (1888), S. 389. See also Stadthagen, *loc. cit.* below.

[6] *Ber. d. d. chem. Gesell.* 1885, Sn. 79, 1928, *Zt. f. physiol. Chem.* Bde. X. (1886), S. 250, XII. (1888), S. 241, XVI. (1892), S. 1. See also Schindler, *Ibid.* XIII. (1889), S. 432. Gives directions for separation of xanthin, hypoxanthin, guanin, and adenin. Thoiss, *Ibid.* Bd. XIII. S. 395. Bruhns, *Ibid.* Bd. XIV. (1890), S. 533. Krüger, *Ibid.* Bd. XVI. (1892), S. 160.

[7] Stadthagen, Virchow's *Arch.* Bd. CIX. (1887), S. 390.

When pure it crystallises in needles from aqueous solutions. Is soluble in 1086 parts of cold water, readily in hot water, insoluble in ether, slightly soluble in hot alcohol. Yields crystalline compounds with acids, also with some salts. The compound with nitrate of silver is soluble in hot nitric acid (sp. gr. 1·1), and is thus separable, together with hypoxanthin, from xanthin. It also yields a readily crystalline compound with picric acid, which is very insoluble in cold water (1 in 3500) and may be used for its quantitative separation from solutions (Bruhns, *loc. cit.*). It does not give the ordinary reactions characteristic of the xanthin bodies, but like hypoxanthin shows a red colouration on the addition of an alkali after treatment with hydrochloric acid and zinc.

7. **Guanin.** $C_5H_5N_5O$.



(Fischer).[1]

It was first obtained from Peruvian guano, which still provides the most convenient source for its preparation.

The guano is finely powdered and boiled with milk of lime as long as it yields a coloured filtrate. The residue is then repeatedly extracted with boiling solution of sodium carbonate; the filtrate on the addition of acetic acid yields a precipitate of guanin and some uric acid, from which it is separated by boiling with somewhat dilute hydrochloric acid. A hydrochloride of guanin is formed which is crystalline, and from this compound the guanin is separated by the addition of concentrated ammonia.[2]

Guanin is also found in small quantities in the pancreas, liver, and muscle extract, and among the products of the action of acids on some nucleins.[3] It may also occur in urine, more especially of pigs, in which case it is also found in many of their tissues;[4] additionally in the retinal tapetum of fishes and in their scales,[5] as also in the integument of amphibia and reptiles,[6] and in vegetable tissues.[7]

It is a white amorphous powder, insoluble in water, alcohol, ether, and ammonia. Its insolubility in the latter distinguishes it from xanthin and hypoxanthin. It unites with acids, alkalis, and salts to form crystallisable compounds. Of its compounds with acids the most characteristic are those with hydrochloric and nitric acids.

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[1] *loc. cit.* (sub xanthin).

[2] Strecker, *Ann. d. Chem. u. Pharm.* Bd. cxviii. (1861), S. 152.

[3] Kossel, *Zt. f. physiol. Chem.* Bd. viii. (1884), S. 404.

[4] Pecile, *Ann. d. Chem. u. Pharm.* Bd. clxxxiii. (1876), S. 141. Cf. Salomon, *Arch. f. Physiol.* 1884, S. 175. *Arch. f. Path. Anat.* Bd. xcv. (1884), S. 527. Virchow, *Arch. f. path. Anat.* Bde. xxxv. (1866), S. 358, xxxvi. S. 147.

[5] Kühne u. Sewall, *Unters. a. d. physiol. Inst.* Heidelb. Bd. iii. (1880), S. 221.

[6] Ewald u. Krukenberg, *Ibid.* Bd. iv. Hft. 3. (1882), S. 253, *Zt. f. Biol.* Bd. xix. (1883), S. 154.

[7] Schulze u. Bosshard, *Zt. f. physiol. Chem.* Bd. ix. (1885), S. 420.

The compound with nitrate of silver is extremely insoluble in strong boiling nitric acid.

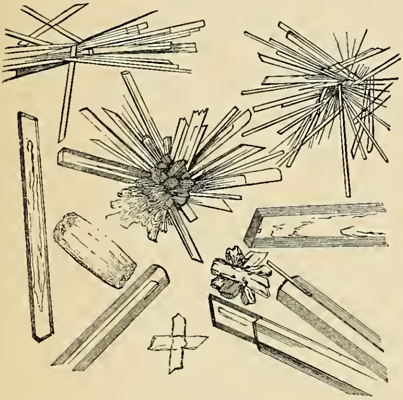

FIG. 29. GUANIN HYDROCHLORIDE, $C_5H_5N_5O \, . \, HCl + H_2O$. (After Kühne.)

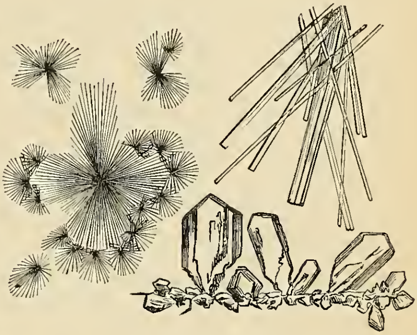

FIG. 30. GUANIN NITRATE, $C_5H_5N_5O \, . \, HNO_3 + 1\frac{1}{2}H_2O$. (After Kühne.)

Reactions. By treatment with nitric acid and caustic soda (Strecker's test) it yields a colouration closely resembling that given by xanthin, but does not respond to Weidel's test (see above, p. 177).

Capranica's reactions.[1] (i) A yellow crystalline precipitate on the addition of a saturated aqueous solution of picric acid to a solution of guanin-hydrochloride; insoluble in cold water. (ii) An orange-coloured crystalline precipitate, very insoluble in water, on the addition of a concentrated solution of potassium chromate. (iii) Prismatic yellowish-brown crystals on the addition of a concentrated solution of ferricyanide of potassium. Xanthin and hypoxanthin when similarly treated do not yield the last two precipitates.

By treatment with nitrous acid guanin may be readily converted into xanthin. (Cf. adenin into hypoxanthin by similar treatment.) By oxidation it yields guanidin $NH : C\,(NH_2)_2$, parabanic acid (see above, p. 171) and carbonic anhydride, a decomposition which obviously corresponds to the formula given above for guanin.

$$C_5H_5N_5O + 3 \, . \, O + H_2O = NH : C(NH_2)_2 + C_3H_2N_2O_3 + CO_2.$$

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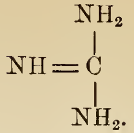
[1] *Zt. f. physiol. Chem.* Bd. IV. (1880), S. 233.

8. **Guanidin.** CN_3H_5.



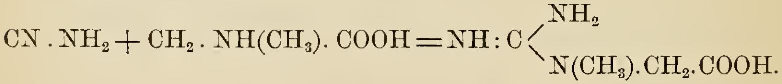
$$\begin{array}{c} NH_2 \\ | \\ NH{=}C \\ | \\ NH_2. \end{array}$$

Although this substance does not occur in the free state in any tissue or fluid of the animal body, it is of considerable interest, for it has been obtained by the direct oxidation of proteids (p. 161) and may be made to yield urea by treatment with boiling dilute sulphuric acid or baryta water. $NH:C(NH_2)_2 + H_2O = (NH_2)_2CO + NH_3$. Further, it affords a connecting link between the xanthin series and kreatin (p. 143), the latter substance being, as already stated, methylguanidin-acetic acid, while guanidin is itself the chief product of the oxidation of guanin.

It may be readily synthetised in several ways; of these its formation by the action of alcoholic ammonia on chlorpicrin (trichlornitromethan) $CCl_3(NO_2)$ or on cyanogen iodide shows clearly its constitution. In the first case

$$CCl_3(NO_2) + 3NH_3 = NH:C(NH_2)_2 + 3HCl + HNO_2.$$

In the second $CNI + 3NH_3 = NH:C(NH_2)_2 + NH_4I$, or in other words guanidin may be regarded as a compound of cyanamide and ammonia $CN.NH_2 + NH_3 = NH:C(NH_2)_2$. The relationship to kreatin may now be at once made evident by comparing the reaction just given with that for the synthesis of kreatin from cyanamide and sarkosin:—



$$CN.NH_2 + CH_2.NH(CH_3).COOH = NH:C\begin{array}{l} \diagup NH_2 \\ \diagdown N(CH_3).CH_2.COOH. \end{array}$$

Xanthin derivatives.

The monomethyl (?) derivative of xanthin (heteroxanthin) has already been described, as also one of the possible dimethyl derivatives, viz. paraxanthin.

When the (silver or) lead salt of xanthin ($PbC_5H_2N_4O_2$) is dried and heated in sealed tubes at 100° with methyl iodide, iodide of lead is formed together with dimethyl-xanthin.[1] The substance thus obtained is identical with theobromin, long known as the characteristic alkaloidal constituent of cocoa-beans, the fruit of *Theobroma cacao*. A third presumably dimethyl derivative of xanthin has recently been described as occurring in tea, viz. theophyllin.[2] When the silver salt of theobromin is further treated as above with methyl iodide it is converted into methyl-theobromin or trimethylxanthin, which is identical with the vegetable alkaloid, long known under the synonymous names of theïne or caffeïne, as occurring in the leaves or seeds of many plants such as tea and coffee, also in the Brazilian 'guarana' prepared from the fruit of *Paulinia sorbilis*, in 'maté' of

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[1] E. Fischer, *loc. cit.* (sub xanthin).

[2] Kossel, *Zt. f. physiol. Chem.* Bd. XIII. (1889), S. 298.

South America, an infusion of the leaves of *Ilex Paraguayensis*, in kola-nuts used as food in Central Africa (the fruit of *Serculia acuminata*), in South African 'bush-tea,' and in many other plants from which stimulating beverages are obtained by infusion.[1] Apart from the close chemical relationship of the alkaloidal principles of the above plants to the nitrogenous crystalline 'extractives' of muscles, it is interesting to notice further that they seem to bear the same general relationship to the organisms in which they respectively occur. There can be but little doubt that the xanthin bodies (and uric acid) are typically products of the downward excretionary nitrogenous metabolism of animals. The alkaloidal principles of plants, in this case theobromin and caffeïne, may be similarly regarded as excretionary products and are hence found collected in those parts of the plant which are more immediately or ultimately cast off, viz. the leaves, seeds, and bark. The facts already stated render the consumption of theobromin and caffeïne in some form or other by practically the whole human race less surprising than it might at first sight appear. Their universal use also indicates that they supply some distinct want of the economy which cannot as yet be explained purely with reference to their relationship to the nitrogenous extractives of animal tissues, but rather to the physiological effect their ingestion produces. In moderate doses they exert an agreeable stimulating action whereby the sensations of fatigue and drowsiness are removed, the body being thus enabled to exert itself with less sense of effort and less initial stimulus, and the mind is more active, clear-sighted and resistent to the depressing effects of unpleasant influences. There is no evidence, as was at one time assumed, that they act in any way by reducing the activity of nitrogenous metabolism.[2] In the case of cocoa and chocolate we have to deal not merely with the stimulating effects of the theobromin they contain, but also with the fact that they are of extreme nutrient value, owing to the large amount of fats (50 p.c.), proteids (12 p.c.), and carbohydrates which enter into their composition. The comparative physiological action of xanthin, theobromin, caffeïne, and some of their derivatives have recently been studied by Filehne.[3]

The Aromatic Series.

1. **Benzoic acid.** $C_6H_5 . COOH$.

This is not found as a normal constituent of the body. When it occurs in (chiefly herbivorous) urine its presence is usually due to a fermentative decomposition of hippuric acid whereby benzoic acid and glycin (glycocoll) are formed.

$$C_6H_5 . CO . NH . CH_2 . COOH + H_2O = C_6H_5 . COOH + CH_2(NH_2) . COOH.$$

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[1] Cf. Johnston and Church, *Chem. of common life*, 1880, p. 147.

[2] Voit, *Unters. üb. d. Einfl. d. Kochsalzes, d. Kaffees, u. s. w.* München, 1860.

[3] *Arch. f. Physiol. Jahrg.* 1886, S. 72. See also Kobert, *Arch. f. exp. Path. u. Pharm.* Bd. xv. (1882), S. 22, and cf. Rossbach, Pflüger's *Arch.* Bd. xxvii. (1882), S. 372.

The acid is usually prepared by the above decomposition of hippuric acid, which is readily effected by a short boiling with mineral acids or, less readily, with caustic alkalis. It is also obtained by the dry distillation of gum-benzoin from which the acid separates by sublimation. The sublimed acid generally crystallises in fine needles which are light and glistening. It is soluble in about 200 parts of cold or 25 of boiling water and very soluble in alcohol, ether, and petroleum-ether,[1] in which latter hippuric acid is insoluble. When precipitated from solutions, either by cooling or the addition of acids to its salts in the cold, the crystalline form is usually much less distinct.

Apart from the crystalline form benzoic acid is characterised by its property of readily subliming, even at 100°, thus resembling leucin and differing markedly from hippuric acid. As a result of this it passes off freely in the vapours arising from its boiling aqueous solutions, so that in concentrating fluids, such as urine, in which its presence is conjectured, they should be first rendered alkaline with sodium carbonate, thus forming a non-volatile salt. Benzoic acid may be additionally recognised by the following test: when treated with a little boiling nitric acid and evaporated to dryness, the residue thus obtained yields, on further heating, an unmistakable odour of nitrobenzol.

When introduced into the body benzoic acid is readily and largely converted into hippuric acid, while at the same time small quantities of succinic acid may at the same time make their appearance. The chief interest in the acid centres in the above relationship to hippuric acid, a fact discovered by Wöhler in 1824 and specially interesting as being the first known instance of a well defined synthesis effected by the animal body, and the starting-point for the disproval of Liebig's views as to the fundamental difference in the metabolic processes of animal and plant tissues.

2. **Hippuric acid.** $C_7H_6O_2$. [$C_6H_5 . CO . NH . CH_2 . COOH.$] (Benzoyl-glycin.)

This acid is found in considerable quantities (1·5 – 2·5 p.c.) in the urine of herbivora, and also, though to a much smaller amount (0·1 – 1·0 grm. per diem) in the urine of man. It is undoubtedly formed in the body by the union, with dehydration, of benzoic acid and glycin (see § 419.) This mode of its formation may be readily observed out of the body by heating together *dry* benzoic acid and glycin in sealed tubes to 160°.

$$C_6H_5.COOH + CH_2(NH_2).COOH = C_6H_5.CO . NH.CH_2.COOH + H_2O.$$

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[1] Petroleum-ether consists ordinarily of a mixture of the more volatile hydrocarbons obtained by distillation during the fractionating of crude petroleum, and boils up to about 120°. The most volatile petroleum-ether boils up to about 80°.

Its constitution is further characteristically shown by its production by the action of benzamide on monochlor-acetic acid:—

$C_6H_5.CO.NH_2+CH_2Cl.COOH=C_6H_5.CO.NH.CH_2.COOH.+HCl.$

and also by that of benzoyl-chloride on glycin:[1]—

$C_6H_5.CO.Cl+CH_2(NH_2).COOH=C_6H_5.CO.NH.CH_2.COOH+HCl.$

It may be readily obtained from the urine of horses or cows, more particularly when they are out to grass,—the *perfectly fresh*[2] urine boiled with milk of lime in slight excess, by which means the acid is fixed as a hippurate of calcium. It is then filtered, the filtrate concentrated to a small bulk and treated when cold with hydrochloric acid in slight excess; this decomposes the calcium salt, liberating hippuric acid, which separates out at once, owing to its comparatively slight solubility. It is then purified by several recrystallisations from boiling water, but it is extremely difficult to obtain it colourless.

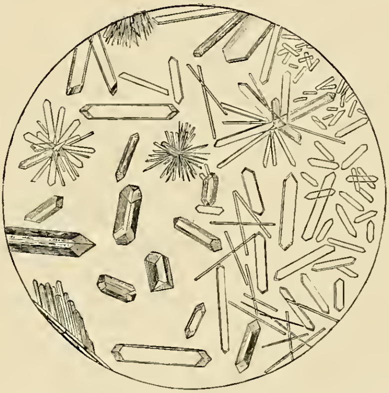

FIG. 31. HIPPURIC ACID CRYSTALS. (After Funke.)

When rapidly separated out from its aqueous solutions, as in the above method of its preparation, it assumes the form of fine needles. By slower crystallisation it yields long foursided prisms or columns with pyramidal ends; these are frequently arranged in groups and present a semitransparent, milky appearance.

When pure they are odourless and of a somewhat bitter taste. They require 600 parts of water for their solution at 0°, are very readily soluble in hot water, also in alcohol and to a less extent in ether. They are conveniently insoluble in petroleum-ether, in virtue of which hippuric acid can be readily separated from benzoic acid which is soluble in this reagent. Its solutions redden litmus-paper.

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[1] Baum, *Zt. f. physiol. Chem.* Bd. IX. (1885), S. 465.

[2] To avoid fermentative decomposition into benzoic acid and glycin.

Hippuric acid is monobasic, and forms salts which (except the iron salts) are readily soluble in water; from these solutions, if sufficiently concentrated, excess of hydrochloric acid precipitates the acid in fine needles. When heated with concentrated mineral acids it is resolved into benzoic acid and glycin. The same decomposition occurs readily in presence of putrefactive organisms.

Apart from the characteristics already stated the acid may be recognised by the following reactions. When *gently* heated in a small tube the acid does not at once sublime as does benzoic acid, but melts and solidifies again on cooling. If more strongly heated it melts as before, but is now decomposed, yielding a sublimate of benzoic acid accompanied by an odour like that of new hay, while oily red drops are observed in the tube. When treated with boiling nitric acid (see above *sub* benzoic acid) and evaporated to dryness the residue on being heated yields the marked and characteristic odour of nitrobenzol (Lücke's reaction [1]). As already stated hippuric acid owes its formation in the body to a union of benzoic acid with glycin, so that its source must be sought for in the modes by which benzoic acid (aromatic substance) is introduced into or arises in the body. The source is probably of more than one kind. Hay and grass were long since stated[2] to contain some substance which yields hippuric acid in the body: this may be extracted by means of dilute sulphuric acid, less readily by caustic potash.[3] More recent researches have shown the presence in grass, hay, and many fruits and berries not only of some benzoic acid but also of substances such as quinic acid $(OH)_4C_6H_7.COOH$, which readily yield benzoic acid and are hence a source of hippuric acid.[4] A further source is found in the aromatic (benzoic) products of the putrefaction of proteids, such as in especial phenyl-propionic acid $(C_6H_5.CH_2.CH_2.COOH)$[5] which in its amidated form is more particularly a product of the decomposition of vegetable proteids,[6] and yields benzoic acid by oxidation. This substance has been found in the rumen of cows fed with hay.[7] These facts coupled with the marked occurrence of putrefactive changes in the alimentary canal of herbivora probably account for the preponderance of hippuric acid in their urine. In carnivora it appears that some traces of hippuric acid may be observed during starvation, originating here from the aromatic residues of the tissue proteids; also during an exclusively meat-diet.[8] When fed on a mixed diet some of the hippuric acid arises from the benzoic and allied constituents of the vegetable part of the food, and probably not an inconsiderable amount from the putrefactive products of the proteids in the alimentary canal; in accordance with this it is found that disinfection of the alimentary canal in dogs with calomel diminishes the output of the acid.[1] Tyrosin, notwithstanding its aromatic constitution, does not give rise to hippuric acid when administered to man.[2]

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[1] *Arch. f. path. Anat.* Bd. XIX. (1860), S. 196.

[2] Meissner u. Shepard, *Die Hippursäure.* 1866.

[3] Weiske, *Zt. f. Biol.* Bd. XII. (1876), S. 241.

[4] For refs. see Salkowski u. Leube. *Die Lehre vom Harn,* 1882, S. 131.

[5] E. u. H. Salkowski, *Zt. f. physiol. Chem.* Bd. VII. (1885), S. 161.

[6] Schulze u. Barbieri, *Ber. d. d. chem. Ges.* 1883, S. 1711. *Jn. f. prakt. Chem.* (N.F.) Bd. XXVII. (1883), S. 337.

[7] Tappeiner, *Zt. f. Biol.* Bd. XXII. (1886), S. 236.

[8] Salkowski, E., *Ber. d. d. chem. Gesell.* 1878, S. 500. *Arch. f. path. Anat.* Bd. 73 (1878), S. 421.

The classical researches of Bunge and Schmiedeberg[3] have shown that the synthetic production of hippuric acid by the union of benzoic acid and glycin takes place chiefly in the kidney of carnivora (dogs). In herbivora (rabbits) it appears that a considerable formation of hippuric acid may be observed on the ingestion of benzoic acid even after exclusion of the kidneys,[4] and the same is the case with frogs. Pathological observations on man seem to indicate that in them the kidneys play at least some part in the synthetic production of hippuric acid from benzoic.[5] When benzoic acid is administered to birds it reappears in the excreta as ornithuric acid: the latter when boiled with hydrochloric acid splits up into benzoic acid and ornithin, the latter having the composition of diamido-valerianic acid.[6]

3. **Tyrosin.** $C_9H_{11}NO_3 . [OH . C_6H_4 . CH_2 . CH . (NH_2) . COOH]$. Para-oxyphenyl-$\alpha$-amidopropionic acid.

The earlier work on the synthesis of tyrosin indicated the probable presence in its molecule of some aromatic (phenyl) radicle. The more recent successful synthesis by the action of nitrous acid on para-amidophenyl alanin[7] has confirmed this view and definitely established its constitution.[8] It always accompanies leucin, though less in amount, as a product of the pancreatic digestion of proteids, but not of gelatin, also as a product of their putrefactive decomposition as well as of the action of boiling mineral acids and alkalis. It is also perhaps found normally in small quantities in the pancreas and its secretion and in the spleen, and *traces* have been described as obtained from various tissues of the body.[9] It is normally absent in urine, but makes its appearance together with leucin in this excretion in several diseased conditions of the liver, notably acute yellow atrophy, also in phosphorus poisoning; there is however some conflict of opinion as to its constancy in such cases. It is also present in not inconsiderable quantities, along with leucin, in many plant tissues.

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[1] Baumann, *Zt. f. physiol. Chem.* Bd. x. (1886), S. 123.

[2] Baas, *Ibid.* Bd. xi. (1887), S. 485.

[3] *Arch. f. exp. Path. u. Pharm.* Bd. vi. (1876), S. 233. Cf. Schmiedeberg, *Ibid.* Bd. xiv. (1881), Sn. 288, 379. See also Hoffmann, A. *Ibid.* Bd. vii. (1877), S. 233.

[4] W. Salomon, *Zt. f. physiol. Chem.* Bd. iii. (1879), S. 365.

[5] Jaarsveld u. Stokvis, *Arch. f. exp. Path. u. Pharm.* Bd. x. (1879), S. 268.

[6] Jaffé, *Ber. d. d. chem. Gesell.* 1877, S. 1925; 1878, S. 406.

[7] Alanin is α-amidopropionic acid; $CH_3 . CH (NH_2) . COOH$.

[8] Erlenmeyer u. Lipp., *Ber. d. d. chem. Gesell.* 1882, S. 1544. Liebig's *Annal.* Bd. 219 (1883), S. 161.

[9] v. Gorup-Besanez, *Lehrb. d. physiol. Chem.* Bd. iv. 1878, pp. 225, 227.

Tyrosin crystallises in exceedingly fine needles which are usually collected into feathery masses. The crystals are snow-white, tasteless, and odourless. If crystallised from an alkaline solution tyrosin often assumes the form of rosettes composed of fine needles arranged radiately.

The crystals are very sparingly soluble in cold water (1 in 2000 at 20°), much more soluble in boiling water (1 in 150); they are almost insoluble in strong alcohol (1 in 13500) and quite insoluble in ether. They are readily soluble in acids and particularly so in ammonia and other alkalis and in solutions of alkaline salts.

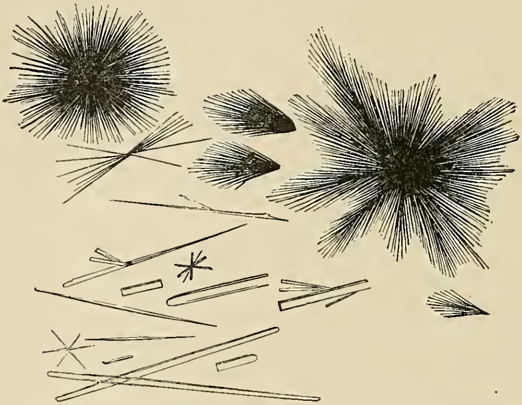

FIG. 32. TYROSIN CRYSTALS. (Krukenberg.)

Preparation. (i) The products of a *prolonged* pancreatic digestion of proteids are neutralised and filtered; the filtrate when concentrated usually yields crusts of tyrosin crystals, which may be readily purified by solution in *a little* boiling water from which they separate out on cooling after concentration if necessary. (ii) Horn shavings are boiled for 24 hours with sulphuric acid (5 of acid to 13 of water). The sulphuric acid is then separated by the addition of lime, and the filtrate from the calcium sulphate yields as before crusts of tyrosin crystals on concentration and cooling. These are then purified by recrystallisation from boiling water.[1] Any leucin at first present in the crystalline crusts remains in the mother-liquors from which the tyrosin has been separated.

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[1] These methods suffice for the preparation of small amounts of tyrosin for purposes of study. For full details of its preparation and most productive separation from leucin see Hlasiwetz and Habermann, quoted sub leucin. See also E. Schulze, *Zt. f. physiol. Chem.* Bd. IX. 1885, Sn. 63, 253, on the separation of amido-acids.

Apart from its crystalline form and characteristic solubilities tyrosin may be readily recognised by several well-marked reactions.

Hoffmann's reaction. When heated with Millon's reagent solutions of tyrosin yield a brilliant crimson or pink colouration which, if much tyrosin is present, is accompanied finally by a similarly coloured precipitate. The test in its original form was applied by heating with a solution of mercuric nitrate in presence of nitrous acid.[1]

Piria's reaction.[2] If tyrosin is moistened on a watch-glass with concentrated sulphuric acid and warmed for five or ten minutes on a water bath, it turns pink, owing to the formation of tyrosin-sulphonic acid — $C_9H_{10}(SO_2OH)NO_3 + 2H_2O$. This is then diluted with water, warmed, neutralised with barium carbonate, and filtered while hot. The filtrate yields a violet colour on the careful addition of very dilute perchloride of iron. The colour is readily destroyed by any excess of the iron salt.[3]

The remarks made on p. 149 on the optical properties of leucin, apply also to tyrosin.[4]

When tyrosin is subjected to putrefactive decomposition it yields paraoxyphenylacetic acid $OH . C_6H_4 - CH_2 . COOH.$, paraoxyphenylpropionic (hydroparacumaric) acid $OH . C_6H_4 - CH_2 . CH_2 . COOH.$, β-phenylpropionic-(hydrocinnamic) acid $C_6H_5 . CH_2 . CH_2 . COOH.$, phenol, $C_6H_5 . OH.$, and parakresol $CH_3 . C_6H_4 . OH.$[5] These substances occur normally in small and variable amounts in urine and are increased in quantity in this excretion by the administration of tyrosin. Their presence is without doubt chiefly due to putrefactive processes occurring in the alimentary canal in correspondence with the facts that the bodies in question are found most markedly in the urine of herbivora, in increased quantity in that of men under a vegetable diet, and largely disappear under the influence of drugs such as calomel, which lessens or prevents the occurrence of putrefactive changes in the intestine.[6] In the absence of these putrefactive processes tyrosin when administered in not excessive amounts is apparently completely oxidised and does not, as frequently stated, give rise to any increased output of urea.[7] In large doses tyrosin reappears externally in the form of tyrosin-hydantoin[1]

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[1] Liebig's *Annal.* Bd. LXXXVII. (1853), S. 124.

[2] Liebig's *Annal.* Bd. LXXXII. (1852), S. 231.

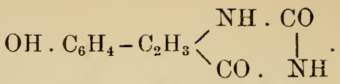
[3] For other less important reactions see Wurster, *Centralb. f. Physiol.* Bd. I. (1887), S. 194. Udránszky, *Zt. f. physiol. Chem.* Bd. XII. (1888), S. 355.

[4] For details see Mauthner, *Monatsb. f. Chem.* Bd. III. (1882), also *Sitzb. d. Wien. Akad.* Bd. LXXXV. (1882), April-Hft. Schulze, *Zt. f. physiol. Chem.* Bd. IX. (1885), Sn. 98, 109. Lippmann, *Ber. d. d. chem. Gesell.* 1884, S. 2838.

[5] Weyl, *Zt. f. physiol. Chem.* Bd. III. (1879), S. 312. Baumann, *Ibid.* Bd. IV. S. 304. Schotten, *Ibid.* Bd. VII. (1882), S. 23. Salkowski, E. u. H. *Ibid.* S. 450. Baumann, *Ibid.* S. 553.

[6] Baumann, *Zt. f. physiol. Chem.* Bd. X. (1886), S. 129.

[7] Schultzen u. Nencki, *Zt. f. Biol.* Bd. VIII. (1872), S. 124. Küssner, *Inaug. Diss.* Königsberg, 1874. Brieger, *Zt. f. physiol. Chem.* Bd. II. (1878), S. 241. Röhmann, *Berl. klin. Wochensch.* 1888. Nrn. 43, 44. Cohn, *Zt. f. physiol. Chem* Bd. XIV. (1819), S. 200.



$$OH \,.\, C_6H_4 - C_2H_3 \left\langle \begin{array}{l} NH \,.\, CO \\ | \\ CO \,.\, NH \end{array} \right. .$$

This substance is the anhydride of tyrosin hydantoic acid[2]

$$OH \,.\, C_6H_4 - C_2H_3\,(NH \,.\, CO \,.\, NH_2)\,COOH.$$

and analogous to the similar compounds excreted after the ingestion of sarkosin and taurin. (See pp. 141, 143.) It yields tyrosin, ammonia, and carbonic dioxide when heated with baryta in sealed tubes.

4. **Kynurenic acid.** $C_{10}H_7NO_3$. [$C_9H_5N \,.\, OH \,.\, COOH$.] Oxychinolin-carboxylic acid.

This acid occurs characteristically but in variable amounts in the urine of dogs, but does not appear to have been found normally in that of man. It was first described by Liebig.[3] It is most readily separated from *fresh* urine by precipitation with phosphotungstic acid after the addition of hydrochloric acid; it is then liberated from the precipitate by the action of baryta.[4] It may also be obtained by concentrating the urine to one-third of its bulk, acidulating with hydrochloric acid and allowing it to stand in a cool place for several days until the separation of the acid is complete.[5] It may be separated from admixed uric acid by solution in dilute ammonia. It is practically insoluble in cold water, slightly so in boiling water, and readily soluble in hot alcohol and

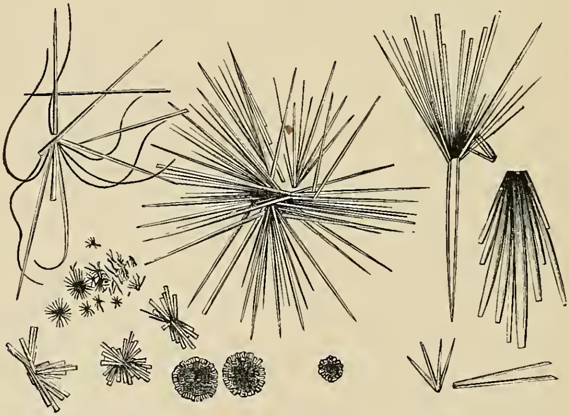


Fig. 33. Crystals of Kynurenic acid. (After Kühne.)

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[1] Blendermann, *Ibid.* Bd. vi. (1882), S. 234.

[2] Jaffé, *Ibid.* Bd. vii. (1883), S. 306.

[3] Liebig's *Annalen*, Bd. 86 (1853), S. 125, Bd. 108 (1858), S. 354.

[4] Hofmeister, *Zt. f. physiol. Chem.* Bd. v. (1881), S. 67. Cf. Brieger, *Ibid.* Bd. iv. S. 89.

[5] Schmiedeberg u. Schultzen, Liebig's *Annalen*, Bd. clxiv. (1872), S. 155.

in dilute ammonia. It crystallises in long brilliant white needles which when kept under acidulated water are often changed into long glittering foursided prisms.

This acid forms salts of which that with barium crystallises readily and in a very characteristic triangular form.

Apart from its crystalline form and that of its barium salt this acid may be readily recognised by the following reaction. When heated on a water bath with hydrochloric acid and chlorate of potash and evaporated to dryness a reddish residue is obtained, which turns at first to a brownish green on the addition of ammonia, and finally to an emerald green.[1]

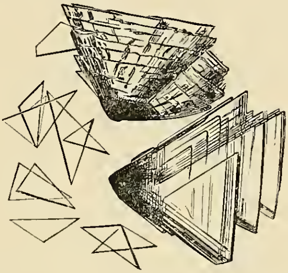

FIG. 34. CRYSTALS OF BARIUM KYNURENATE. (After Kühne.)

By prolonged heating to 250—260° kynurenic acid evolves carbonic anhydride and is converted into kynurin (oxychinolin) $C_9H_6N(OH)$, and when heated with zinc dust in a current of hydrogen it is converted into chinolin $C_9H_6N(OH) + H_2 = C_9H_7N + H_2O$. These reactions throw considerable light on the constitution of the acid.[2]

The amount of kynurenic acid in the urine is increased on the ingestion of isatin, a product of the oxidation of indigo.[3] Under ordinary conditions its amount in this excretion is dependent upon the nature of the food supplied to the animal, being greatest under a proteid diet, and is not related to the occurrence or absence of putrefactive processes in the alimentary canal.[4]

5. **Phenol.** $C_6H_5.OH$. Oxybenzol. (Carbolic or phenylic acid.)

This substance is formed, together with indol and skatol, during the putrefactive decomposition of proteids, more especially in prolonged putrefactive pancreatic digestions.[5] From these it may be obtained by simple distillation. In accordance with this it is formed in not inconsiderable quantity in the alimentary canal, more especially when putrefactive processes in its contents are increased either pathologically or as the result of experimental interference.[1] On the phenol thus formed a small proportion is passed out in the fæces,[2] the larger part however is excreted in the urine as an ethereal salt of sulphuric acid, viz. phenylsulphate of potassium. The latter is typical of an extensive series of similar ethereal sulphates which make their appearance in urine after the ingestion of aromatic substances.

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[1] Jaffé, *Zt. f. physiol. Chem.* Bd. VII. (1882–3), S. 399.

[2] Kretschy, *Ber. d. d. chem. Gesell.* Bd. XII. (1879), S. 1673. *Monatsh. f. Chem.* Bd. II. (1881), S. 57.

[3] Niggeler, *Arch. f. exp. Path. u. Pharm.* Bd. III. (1874), S. 67.

[4] Baumann, *Zt. f. physiol. Chem.* Bd. X. (1886), S. 131. But cf. Haagen, Inaug.-Diss., Königsb., 1887. (See *Centralb. f. d. Med. Wiss.* 1889, S. 214.)

[5] Baumann, *Zt. f. physiol. Chem.* Bde. I. (1877), S. 60, III. 250. Brieger, *Ibid.* Bd. III. (1879), S. 134. Odermatt, *Jn. f. prakt. Chem.* Bd. XVIII. (1878), S. 249.

Their nature and constitution was first definitely ascertained by Baumann,[3] although it had previously been shown that phenol, even after it has been administered as such, does not exist in the free state in urine but may be set free by distillation with a mineral acid.[4]

Phenyl-sulphuric acid.[5] $C_6H_5 . O . SO_2OH$. Apart from its abundant presence in urine as an alkaline salt after the administration of phenol this compound occurs normally in small quantities in most urines, more particularly in those of herbivora, since in these animals the conditions for its formation are especially provided by the preponderance of aromatic compounds in their food and the more marked activity of putrefactive changes in their alimentary canal. The total sulphates in urine consist therefore partly of this ethereal sulphate (together with the similar compounds of kresol, indol, and skatol, *see below*) and of ordinary sulphates. The relative amounts of the sulphuric acid contained in these two forms is ascertained by acidulating with acetic acid and adding barium chloride, by which the sulphuric acid present as ordinary sulphates is precipitated as barium sulphate. The filtrate from this is now boiled with hydrochloric acid, by whose action the ethereal sulphates are decomposed, yielding phenol and sulphuric acid, which again forms barium sulphate; from this the amount of the ethereal salts of sulphuric acid may be at once determined.[6] While the probable mode of

[1] E. Salkowski, *Ber. d. d. chem. Gesell.* 1876, S. 1595. *Ibid.* 1877, S. 842. *Centralb. f. d. Med. Wiss.* 1876, S. 818. *Arch. f. Physiol.* Jahrg. 1877, S. 476. Brieger, *Zt. f. physiol. Chem.* Bd. II. (1878), S. 241. G. Hoppe-Seyler, *Ibid.* Bd. XII. (1888), S. 1.

[2] Brieger, *Ber. d. d. chem. Gesell.* 1877, S. 1027. *Jn. f. prakt. Chem.* Bd. XVII. (1878), S. 134.

[3] Pflüger's *Arch.* Bd. XIII. (1876), S. 285. *Ber. d. d. chem. Gesell.* 1876, S. 55. Baumann und Herter, *Zt. f. physiol. Chem.* Bd. I. (1877), S. 244. See also Baumann, *Ibid.* Bd. II. (1878), S. 335, Bd. x. (1886), S. 123. For a list of substances which when administered leave the body as ethereal sulphates, see Hermann's *Hdbch. d. Physiol.* Bd. v. Th. 1, S. 508.

[4] Buliginsky, Hoppe-Seyler's *Med. chem. Unters.* Hft. 2, 1866, S. 234. Hoppe-Seyler, Pflüger's *Arch.* Bd. v. (1872), S. 470.

[5] Not to be confounded with phenolsulphonic acid, $C_6H_4 (OH) . SO_2 OH$.

[6] For the accurate separation of the ethereal sulphates which usually occur mixed in urine, some special works should be consulted, such as Neubauer u. Vogel, *Analyse des Harns*, or Salkowski u. Leube, *Die Lehre vom Harn.* Cf. Baumann, *Zt f. physiol. Chem.* Bd. I. (1876), S. 70, *Ibid.* Bd. VI. (1882), S. 183.

formation of this acid is undoubtedly due to the primary production of phenol by putrefactive processes from proteids, and the subsequent colligation of this phenol with sulphuric acid, very little is known of the seat or mode of this union. It has not been definitely connected, if at all, with any distinctly synthetic activity of the kidney.[1]

Since, as has been said, phenol does not exist in the free state in urine, its detection necessitates the decomposition of its compound, viz. the phenylsulphate of potassium. This is best brought about by distilling the urine (200 c.c.) with strong hydrochloric acid (40 c.c.) or 5 p.c. of sulphuric acid until about 150 c.c. of distillate has passed over. The distillate contains free phenol, which is tested for qualitatively by the reactions described below, and estimated quantitatively by the formation of a compound with bromine, tribromphenol, $C_6H_2Br_3 . OH$.[2]

Phenol reactions (i). A violet-blue colouration on the addition of neutral solutions of perchloride of iron. This colour is similar to that yielded by salicylic acid, but the absorption spectra of the two are stated to be different.[3] It is destroyed by excess of the reagent and is also not obtained in presence of acids and alkalis or of alcohol.[4] (ii) When a solution of phenol is mixed with one quarter of its bulk of ammonia and a few drops of chloride of lime solution (1 to 20 of water) and gently warmed it yields a blue colouration.[5] (iii) When boiled with Millon's reagent a marked and persistent pink or red colour similar to that yielded by tyrosin is obtained.[6] (iv) Mere traces of phenol give a yellowish crystalline precipitate on the addition of bromine water. This reaction is used as stated above for the quantitative estimation of phenol. Of these reactions (iii) and (iv) are the most delicate. (v) On the addition of furfurol ($C_5H_4O_2$, aldehyde of pyromucic acid) solution (·5 p.c.) and strong sulphuric acid, phenol yields a brilliant red colour which finally turns to blue.[7]

6. **Kresol.** $C_6H_4 . OH . CH_3$. Methylphenol.

This homologue of phenol exists in three isomeric forms, ortho-, para-, and metakresol. It is now known that the phenols which may be obtained by the distillation of urine with acids consist preponderatingly of parakresol, accompanied in some cases by orthokresol and possibly (?) by metakresol in minute amounts. Like phenol it is not found free in urine, but as kresylsulphuric acid,[1] $C_7H_7O . SO_2OH$. The general conditions of its presence in urine are practically identical with those for the occurrence of phenylsulphuric acid.[2] When introduced into the animal body the three isomeric kresols undergo distinctly different oxidational changes.[3]

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[1] Christiani u. Baumann, *Zt. f. physiol. Chem.* Bd. II. (1878), S. 350. See also Kochs, Pflüger's *Arch.* Bd. XX. (1879), S. 64.

[2] Landolt, *Ber. d. d. chem. Gesell.* 1871, S. 770.

[3] Krukenberg, *Verhandl. d. physik.-med. Gesell. zu Würzburg*, Bd. XVIII. (1884), S. 197.

[4] Hesse, Liebig's *Annal.* Bd. 182 (1876), S. 161.

[5] E. Salkowski, Pflüger's *Arch.* Bd. V. (1872), S. 353.

[6] Plugge, *Zt. f. anal. Chem.* Bd. XI. (1872), S. 173 See also Almén, *Ibid.*, Bd. XVII. (1878), S. 107.

[7] Udránszky, *Zt. f. physiol. Chem.* Bd. XII. (1888), Sn. 355, 377.

Reactions. On the addition of an excess of bromine water to its solutions parakresol yields a brominated derivative, but the compound is only obtained in a separate and crystalline form after prolonged standing, differing characteristically from the analogous compound of phenol, which under similar circumstances is formed rapidly. It yields a reddish yellow colouration with potassium nitroprusside and caustic potash, which turns bright pink on the addition of an excess of acetic acid.[4] Aceton gives a similar reaction. With furfurol and sulphuric acid the reaction is closely similar to that which phenol gives.[5]

7. **Pyrocatechin.** $C_6H_4 (OH)_2$. Orthodioxybenzol.

This substance occurs, in small amounts in human urine united with sulphuric acid as a mono-ethereal compound $OH . C_6H_4 . O . SO_2OH$. It is more plentifully present in the urine of herbivora, especially of the horse, and is largely increased in amount by the administration of benzol or phenol.[6] It is also stated to occur in cerebrospinal fluid.[7] When present in urine it (together with hydrochinon) confers on this excretion, especially if alkaline, the property of turning successively greenish, brown, and finally dark-brown or almost black on exposure to the air, and of readily reducing solutions of metallic salts, a fact to be taken into account when dealing with the presence or absence of sugar in the urine. Solutions of pyrocatechin turn emerald green on the addition of a few drops of very dilute solution of ferric chloride, avoiding all excess of the reagent. If the green solution is now acidulated with tartaric acid, it turns violet on the subsequent addition of a little ammonia and purplish-red on the addition of excess. The green colour may be restored by excess of acetic acid.[8] It may be distinguished from hydrochinon by yielding a precipitate with normal acetate of lead which is soluble in acetic acid, whereas the latter substance does not. No simple directions can be given for the separation and estimation of pyrocatechin in presence of phenol, kresol, and hydrochinon.[1]

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[1] Baumann, *Ber. d. d. chem. Gesell.* Bd. IX. (1876), S. 1389. *Zt. f. physiol. Chem.* Bd. II. (1878), S. 335. Preusse; *Ibid.* S. 355. Brieger, *Ibid.* Bd. IV. (1880), S. 204.

[2] Baumann u. Brieger; *Ibid.* Bd. III. (1879), S. 149. Baumann, *Ibid.* IV. S. 304. For the detection and separation of the kresols and phenol see Baumann u. Brieger, *Ber. d. d. chem. Gesell.* Bd. XII. (1879), S. 804. Baumann, *Zt. f. physiol. Chem.* Bd. VI. (1882), S 183. Brieger, *Ibid.* VIII. (1883), S. 311.

[3] Preusse, *Ibid.* Bd. V. (1881), S. 57.

[4] v. Jacksch, *Zt. f. klin. Med.* Bd. VIII. (1884), S. 130.

[5] Udránszky, *cit.* (sub phenol).

[6] See Baumann, Pflüger's *Arch.* Bd. XII. (1876), S. 63, Baumann u. Herter, *Zt. f. physiol. Chem.* Bd. I. (1877), S. 248, Baumann u. Preusse, *Ibid.* Bd. III. (1879), S. 156. Brieger, *Arch. f. physiol. Jahrg.* 1879, Suppl.-Bd. S. 61. Nencki u. Giacosa, *Zt. f. physiol. Chem.* Bd. IV. (1880), S. 325. Schmiedeberg, *Arch. f. exp. Path. u. Pharm.* Bd. XIV. (1881), S. 288.

[7] Halliburton, *Jl. of Physiol.* Vol. X. (1889), p. 247.

[8] Ebstein u. Müller, Virchow's *Arch.* Bd. LXV. (1875), S. 394. See also Jacquemin, *Rev. Méd. de l'Est.* T. VIII. (1877), p. 90.

But little is known as to the source of this substance in urine apart from its probable formation from the phenol produced by putrefactive changes in the alimentary canal. In herbivora there is some evidence that it is derived from certain aromatic constituents of their food.[2]

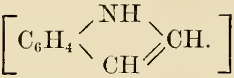
8. **Hydrochinon.** $C_6H_4\,(OH)_2$. Paradioxybenzol.

Has not yet been described as occurring normally in urine, but only as the result of the ingestion of phenol. It exists in urine as an ethereal compound with sulphuric acid, and is largely the cause of the dark colour which this excretion assumes after the absorption of phenol on exposure to the air. It resembles pyrocatechin in effecting the reduction of metallic salts, but differs from it in being nearly insoluble in cold benzol and in not yielding any precipitate with normal lead acetate. This latter property suffices for its separation from pyrocatechin. It is readily converted by oxidation into chinon $C_6H_4O_2$ whose characteristic odour affords a further means of identification, and when heated in an open test-tube it yields a blue sublimate.[3]

The third known isomeric dioxybenzol, viz. meta-dioxybenzol or resorcin, has not yet been found in the animal body or in urine.

THE INDIGO SERIES.

1. **Indol.** C_8H_7N.



$$\left[C_6H_4 \left\langle \begin{matrix} NH \\ CH \end{matrix} \right\rangle CH.\right]$$

Indol occurs characteristically in the fæces, to which with skatol it imparts their peculiarly unpleasant odour.[4] Its presence here is due to its formation during the putrefactive decomposition of proteids which usually occurs to a greater or less extent in the alimentary canal, part of the indol leaving the body in the urine as a potassium salt of indoxylsulphuric acid (*see below*), the remainder being excreted with the fæces. It may readily be obtained, contaminated by varying quantities of phenol and skatol (*see below*), by acidulating and distilling the products of a not too prolonged alkaline *putrefactive* pancreatic digestion of proteids, preferably of liver or fibrin. Indol passes over into the distillate, from which it is extracted by shaking up with ether, and is left behind as an impure oily liquid when the ether is driven off by heat.[1] It may also be prepared by heating moist proteids slowly to a red-heat with excess of caustic potash, the indol as before passing over into the distillate.[2] Indol is a crystalline body which when pure melts at 53°. It is soluble in boiling water, alcohol, and ether.

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[1] See Baumann, *Zt. f. physiol. Chem.* Bd. VI. (1882), S. 183. Schmiedeberg, *loc. cit.* S. 304.

[2] Preusse, *Zt. f. physiol. Chem.* Bd. II. (1878), Sn. 324, 329.

[3] In addition to the literature precedingly quoted, see more particularly Baumann u. Preusse, *Arch. f. physiol. Jahrg.* 1879, S. 245. Brieger, *Ibid.* Suppe-Hft. S. 66. Baumann u. Preusse, *Zt. f. physiol. Chem.* Bd. VII. (1889), S. 156 Baumann, *Ibid.* Bd. VI. (1882), S. 188.

[4] Radziejewski, *Arch. f. Anat. u. Physiol.* 1870, S. 42.

Reactions. A strip of pine-wood moistened with hydrochloric acid is coloured bright crimson when dipped into an alcoholic solution of indol.[3] Its alcoholic solution turns red when treated with nitrous (fuming nitric) acid, and its aqueous solution gives a copious red precipitate with the same reagent.[4] This reaction is more delicate if carried on by the addition of strong nitric acid first, and of a 2 p.c. solution of potassium nitrite subsequently.[5] When indol in dilute solution is mixed with a little sodium nitroprusside and then with a few drops of caustic soda it turns at once violet-blue, and pure blue on subsequent acidulation with acetic acid.[6] Skatol yields neither of the above reactions. Indol also forms a well-marked crystalline compound with picric acid (trinitro-phenol) when added in benzolic solution to a solution of the acid in benzol, so also does skatol.

It has been already stated that a part of the indol formed in the alimentary canal leaves the body in the urine as a potassium salt of indoxylsulphuric acid; by oxidation this may be readily decomposed into indigo-blue and acid potassium sulphate:—$2C_8H_6NKSO_4 + O_2 = C_{16}H_{10}N_2O_2 + 2KHSO_4$.[7] By the action of powerful reducing agents indigo-blue may be made to yield indol, which by oxidation may be again converted into indigo-blue. This shows that indol is the mother substance of the indigo series. The constitution of indol is elucidated by its formation from orthonitrophenylchlorethylene $C_6H_4(NO_2)-CH=CHCl$. When this is reduced with tin and hydrochloric acid it yields $C_6H_4(NH_2)-CH=CHCl$, and this when heated to 160°—170° with sodium-ethylate ($NaO.C_2H_5$) yields sodium chloride, ethyl-alcohol and indol.[8]

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[1] Nencki, *Ber. d. d. chem. Gesell.* Bde. vii. (1874), S. 1593, viii. S. 336, 722. Brieger, *Zt. f. physiol. Chem.* Bd. iii, (1879). S. 134. Cf. Koukol-Yasnopolsky, Pflüger's *Arch.* Bd. xii. (1876), S. 78. Baumann, *Zt. f. physiol. Chem.* Bd. i. (1877), S. 63. Weyl, *Ibid.* S. 339. See specially E. Salkowski, *Ibid.* Bd. viii. (1884), S. 417.

[2] Kühne, *Ber. d. d. chem. Gesell.* Bd viii. (1875), S. 206. Nencki, *Jn. f. prakt. Chem.* (N. F.), Bd. xvii. (1878), S. 97.

[3] This reaction depends on the presence of coniferin in the pine-wood. Phenol under similar conditions yields a blue colouration. But see Udránszky, *Zt. f. physiol. Chem.* Bd. xii. (1888), S. 367.

[4] Cf. Nencki, *Ber. d. d. chem. Gesell.* Bd. viii. (1875), S. 722.

[5] E. Salkowski, *loc. cit.*

[6] Legal, *Bresl. ärtzl. Zeitsch.* Nrn. 3 u. 4, 1883.

[7] Baumann u. Brieger, *Zt. f. physiol. Chem.* Bd. iii (1879), S. 254.

[8] Lipp, *Ber. d. d. chem. Gesell.* Bd. xvii. (1884), S. 1067.

2. **Indoxylsulphuric acid.** $C_8H_6N . O . SO_2OH$. The indican of urine.

A substance was long ago described as frequently occurring in the urine and sometimes in the sweat of man and other animals which yielded by the action of acids the blue colouring matter indigo as one of the products of its decomposition. It was regarded at that time as identical with the indican known to occur in several plants (Indigofera tinctoria, Isatis tinctoria). Hoppe-Seyler on the other hand, having regard to the greater ease with which the indican of plants undergoes decomposition, regarded them as most probably different substances.[1] This view was confirmed by the researches of Baumann, who first proved that urinary indican is not a glucoside, as is that of plants, but is in reality an ethereal compound of sulphuric acid with indoxyl ($C_8H_6N . OH$) analogous to those already described above as derived from phenol, kresol, &c.[2] Indol, as previously stated, is a characteristic product of the putrefaction of proteids. Further, when administered to animals, it leads to a correspondingly increased output of urinary indican,[3] an increase which is similarly observed as the result of either a normally, pathologically, or experimentally increased activity of putrefactive processes in the alimentary canal.[4] Hence indican is under normal conditions more plentiful in the urine of herbivora than of carnivora. It is also increased in carnivorous urine under a meat diet, is not increased by the administration of gelatin and is least during starvation, although in the latter case it may not entirely disappear.[5] These facts correspond again to the experimental observations that gelatin does not yield indol during its putrefactive decomposition,[6] whereas mucin does,[7] and the latter substance constitutes a part at least of the contents of the alimentary canal during starvation. These statements show clearly the origin and mode of formation of urinary indican, the first-formed indol undergoing oxidation into indoxyl, which is subsequently united to the elements of sulphuric acid and excreted as an ethereal compound.

Indoxyl-sulphuric acid is not known in the free state; its most important salt is that with potassium, the form in which it occurs in urine.[1] When warmed in aqueous solution with hydrochloric acid it decomposes into indoxyl and potassium sulphate:—

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[1] For earlier literature see Hoppe-Seyler's *Physiol.-path. chem. Anal.* Aufl. 4, 1875, S. 191; and *Physiol. Chem.* 1881, S. 841.

[2] Pflüger's *Arch.* Bd. XIII. (1876), S. 301; *Zt. f. physiol. Chem.* Bd. I. (1877), S. 60; III. (1879), S. 254. Cf. G. Hoppe-Seyler, *Ibid.* Bde. VII. (1883), S. 403; VIII. S. 79.

[3] Jaffé, *Centralb. f. d. med. Wiss.* 1872, Sn. 2, 481, 497. Virchow's *Arch.* Bd. LXX. (1877), S. 72.

[4] Jaffé, *loc. cit.* Ortweiler, *Mittheil. d. Würzburg. med. Klinik.* Bd. II. (1886), S. 153. Gives literature to date.

[5] Fr. Müller, *Ibid.* S. 341; *Berl. klin. Wochensch.* 1887, Nr. 24. (Results of experiments on Cetti.)

[6] Nencki, *Ber. d. d. chem. Gesell.* Bd. VII. (1874), S. 1593. See also Abst. in Maly's *Jahresb.* 1876, S. 31. Weyl, *Zt. f. physiol. Chem.* Bd. I. (1877), S 339.

[7] Wälchli, *Jn. f. prakt. Chem.* (N.F.), Bd. XVII. (1878), S. 71.

$$C_8H_6N.O.SO_2.OK + H_2O = C_8H_6N(OH) + KHSO_4.$$

Indoxyl by oxidation is converted into indigo-blue:—

$$2C_8H_6N(OH) + O_2 = C_{16}H_{10}N_2O_2 + 2H_2O.$$

The blue colouration which results from the above reaction affords the one test for the presence of indican in urine. The test is applied as follows (Jaffé). A small volume of urine (10 c.c.) is mixed with an equal volume of strong hydrochloric acid and 2—3 c.c. of chloroform. A strong solution of chloride of lime is then added drop by drop, shaking after the addition of each drop. If indican is present the layer of chloroform which settles on standing will be coloured more or less brilliantly blue according to the amount of indican in the urine.[2] The formation of indigo-blue is also the basis for the quantitative estimation of indican. The latter is converted into indigo-blue by oxidation and the indigo-blue is estimated either by weighing or colorimetrically or spectrophotometrically.[3]

3. **Indigo-blue.** $C_{16}H_{10}N_2O_2$.

It is formed, as stated above, from indican, and gives rise to the bluish colour sometimes observed in sweat and urine.

It may, by slow formation from indican, be obtained in fine crystals; these are insoluble in water, slightly soluble, with a faint violet colour, in alcohol and in ether. Chloroform dissolves them to a slight extent, as also does benzol. Indigo is soluble in strong sulphuric acid, forming at the same time two compounds with the acid, indigo mono- and di-sulphonic acids. The sodium salts of these acids are soluble in water and, when mixed with sodium sulphate, constitute the crude 'indigocarmine' of commerce, and in a purer form the sulphindigotate of soda used in certain experiments on the nature of the excretory activity of the kidney and other glands (see § 416). These soluble sulphonates give an absorption band in the spectrum which lies to the red side of and close to the D line. This may be used to detect indigo.

Indigo as ordinarily seen possesses a pure blue colour; it leaves a reddish copper-coloured mark when pressed with a hard body, and the crystals exhibit the same colour if seen in reflected light.

Treated with reducing agents in strongly alkaline solution indigo is decolourised, being reduced to indigo-white. The latter contains two atoms of hydrogen more than indigo, is reconverted into indigo-blue by exposure to the air, and thus provides a convenient reaction for the detection of either indigo or of reducing substances such as dextrose.

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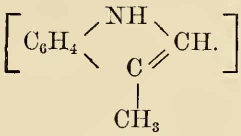
[1] For its isolation and preparation from urine see Baumann u. Brieger, *Zt. f. physiol. Chem.* Bd. III. (1879), S. 254. See also Baumann u. Tiemann, *Ber. d. deutsch. Chem. Gesell.* XII. (1879), Sn. 1098, 1192; and XIII. (1880), S. 408.

[2] Jaffé, Pflüger's *Arch.* Bd. III. (1870), S. 448. Cf. Senator, *Centralb. f. d. med. Wiss.* 1877, S. 357.

[3] For details and literature see Neubauer u. Vogel, *Die Harnanalyse*, 1890, S. 492.

4. **Skatol.** C_9H_9N.



Methyl-indol.

Skatol was first noticed and definitely described by Brieger as occurring in human fæces together with indol, the latter usually being less in amount than the former.[1] Later researches have shown that the conditions of its production are in general the same as those for the formation of indol, so that the two substances occur mixed in variable proportions among the products of the putrefactive decomposition of proteids[2] or brain-substance[3] and of the action of caustic potash at high temperatures on proteids.[4] In the former case it appears to be produced at a later stage than is indol, so that on the whole it is most preponderant the longer the putrefactive change is allowed to proceed. Its presence in the fæces is thus due to causes similar to those which account for the presence of indol, and the resemblance is further shown by the fact that a portion of the first-formed skatol is absorbed, oxidised, and appears externally in the urine as skatoxyl-sulphuric acid (*see below*).

Skatol is formed in small quantities during the preparation of indol by reduction from indigo.[5] It may be partly converted into indol by passing its vapours through a red-hot porcelain tube.[6] The constitution of skatol was foreshadowed by its preparation from the barium salts of ortho-nitrocuminic acid, $(CH_3)_2CH . C_6H_3(NO_2) . COOH$[7] and clearly proved by its synthetic production from propylidene-phenylhydrazin $C_6H_5 . NH . N = CH . CH_2 . CH_3$, the product of the action of propionic aldehyde $(CH_3 . CH_2 . COH)$ on phenylhydrazin $(C_6H_5 . NH.$

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[1] *Ber. d. d. chem. Gesell.* Bd. x. (1877), S. 1027. *Jn. f. prakt. Chem.* (N.F.), Bd. xvii. (1878), S. 124. A closely similar, if not identical, substance had previously been noticed, but not clearly characterised, by Nencki, as among the products of the putrefactive decomposition of gelatin, and by Sécretan among those of a similar decomposition of proteids. See Maly's *Jahresb.* 1876, Sn. 31, 39.

[2] Nencki, *Centralb. f. d. med. Wiss.* 1878, S. 849. E. u. H. Salkowski, *Ber. d. d. chem. Gesell.* Bd. xii. (1879), S. 648. *Zt. f. physiol. Chem.* Bd. viii. (1884), S. 417—466. Contains very full references to previous literature.

[3] Nencki, *Ibid.* Bd. iv. (1880), S. 371. Stöckly, *Jn. f. prakt. Chem.* (N.F.), Bd. xxiv. (1881), S. 17.

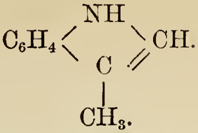
[4] Nencki, *Jn. f. prakt. Chem.* (N.F.), Bd. xvii. (1878), S. 97.

[5] Baeyer, *Ber. d. d. chem. Gesell.* Bd. xiii. (1886), S. 2339.

[6] Fileti, *Gazz. Chim.* T. xiii. (1883), p. 378. See abstr. in *Ber. d. d. chem. Gesell.* 1883, S. 2928.

[7] Fileti, *loc. cit.* p. 356. Abst. *loc. cit.*, S. 2927.

NH_2). When this substance is heated with zinc chloride it loses NH_3 and yields skatol [1]



Since the condition of the occurrence and formation of skatol are on the whole the same as those for indol, and since these substances further resemble each other in being both volatile and hence passing over in the vapours arising from their heated solutions, the method previously described for the preparation of indol from putrefactive products may be applied for the preparation of skatol. The separation of the two depends chiefly on the fact that skatol is much less soluble in water than is indol, so that if the mixed substances are dissolved in a minimal amount of absolute alcohol, then on the addition of 8 — 10 volumes of water, indol remains in solution while skatol is precipitated.[2] Skatol is unaffected by being boiled with moderately strong caustic soda, whereas indol is decomposed. This difference in behaviour to caustic alkalis provides a further means by which the former may be obtained free from the latter.

Skatol is a crystalline substance which melts when heated to 93°, and has a powerfully unpleasant odour, somewhat like that of indol.

Reactions. Many of the reactions of skatol resemble so closely those of indol that they provide no means for distinguishing between the two substances. Skatol is however characterised by yielding only a milky opacity instead of a red colouration on the addition of fuming nitric acid to its aqueous solutions (*see sub* indol), in not giving the reaction with a strip of pine-wood dipped in hydrochloric acid which indol does,[3] by its lesser solubility in water and greater resistance to the action of caustic soda.

5. **Skatoxyl-sulphuric acid.** $C_9H_8N . O . SO_2OH$.

The close relationship between indol and skatol is further shown by the fact that the latter, like the former, after absorption from the alimentary canal is oxidised, the product being skatoxyl $C_9H_8N . OH$, which unites, as does indoxyl, with the elements of sulphuric acid and leaves the body in the urine as a potassium salt of the above acid.[4] This salt may be isolated from urine by methods similar to those used for the preparation of indoxyl-sulphuric acid.

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[1] E. Fischer, *Ber. d. d. chem. Gesell.* Bd. XIX. (1886), S. 1563. Liebig's *Ann.* Bd. CCXXXVI. (1886), S. 116.

[2] Brieger, *Ber. d. d. chem. Gesell.* Bd. XII. (1879), S. 1985. *Zt. f. physiol. Chem.* Bd. IV. (1880), S. 414.

[3] When, however, a strip of pine-wood is dipped into an *alcoholic* solution of skatol and subsequently into strong hydrochloric acid, it is coloured first crimson, which turns to bluish violet. Fischer, Liebig's *Ann.* Bd. CCXXXVI. (1886), S. 140.

[4] Brieger, *Zt. f. physiol. Chem.* Bd. IV. (1880), S. 414. Baumann u. Brieger, *Ibid.* Bd. III. (1879), S. 255. G. Hoppe-Seyler, *Ibid.* Bd. VII. (1883), S. 423.

Our knowledge of the quantitative formation of skatol in the alimentary canal and of its relationship to the simultaneous production of indol is far less complete than is that respecting the latter substance. Notwithstanding the close chemical relationship of the two it appears that their physiological behaviour is markedly different. In the first place it seems that the absorption of skatol is less complete than that of indol, since it preponderates in the normal fæces:[1] in accordance with this but little of its ethereal sulphate is found normally in urine.[2] Further, whereas by the ingestion of indol nearly the whole of the sulphates of the urine may be converted into the ethereal compound with indoxyl, when skatol (synthetically prepared) is similarly employed a large part reappears in the fæces; and although at first the ethereal sulphates are increased, they subsequently diminish even with continued injection of skatol, and are stated to finally disappear. Indoxyl-sulphuric acid may be regarded as a urinary chromogen, since it yields a pigment, indigo, by oxidational decomposition; so also may skatoxyl-sulphuric acid, but it is found that the amount of pigment-forming material specifically present in the urine of a dog fed with skatol is not so directly proportional to the amount of skatoxyl-sulphuric acid as it is to the similar compound of indoxyl when indol is administered. It has been suggested that a large part of the skatolic chromogen exists as a compound of skatoxyl and glycuronic acid.[3] When Jaffé's test (see p. 200) for urinary indican is applied to urine which contains skatoxyl compounds the results obtained are as follows. The urine turns dark red or violet on the addition of hydrochloric acid, bright crimson on the addition of nitric acid, and a similar colour is obtained if it is warmed with hydrochloric acid and ferric chloride. The colouring matter thus obtained is probably formed from the skatoxyl (not known in the free state), and by reduction may be made to yield skatol.

Skatol has recently been described as occurring in a vegetable tissue, namely the wood of an East Indian tree, Celtis reticulosa.[4]

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[1] It is absent from the fæces of the dog.

[2] The chief record of its occurrence is in a case of diabetes mellitus with gastric disturbance. Otto, Pflüger's *Arch.* Bd. XXXIII. (1884), S. 607.

[3] Mester, *Zt. f. physiol. Chem.* Bd. XII. (1888), S. 130. A similar compound of indoxyl with glycuronic acid has been described. Schmiedeberg, *Arch. f. exp. Path. u. Pharm.* Bd. XIV. (1881), S. 306. To complete the literature of this substance see E. Salkowski, *Zt. f. physiol. Chem.* Bd. VIII. S. 417; IX. (1884), Sn. 8, 23.

[4] Dunstan, *Pharm. Jl.* Vol. XIX. (1889), p. 1010. *Ber. d. d. chem. Gesell.* (Referate), Bd. XXII. (1889), S. 441. *Proc. Roy. Soc.* Vol. XLVI. (1889), p. 211.

The Ptomaines.

The now extensive literature of these substances may be most conveniently and inclusively indicated by reference to the following publications. Selmi (to whom the name ptomaine is due), *Sulle ptomaine od alcaloidi cadaverici.* Bologna, 1878. Gautier, *Compt. Rend.* T. xciv. (1882), p. 1119. Guareschi e Mosso, *Arch. ital. de Biol.* T. ii. (1883), p. 367; iii. (1883), p. 241. Abstr. in *Jn. f. prakt. Chem.* (N.F.), Bd. xxvii. S. 425; xxviii. S. 504. Brieger, *Zt. f. physiol. Chem.* Bd. vii. (1883), S. 274. *Ber. d. d. chem. Gesell.* Bd. xvi. (1883), Sn. 1186, 1405. E. u. H. Salkowski, *Ibid.* S. 1191. Brieger, *Ibid.* Bd. xvii. (1884), Sn. 515, 1137, 2741; xix. (1886), S. 3119. *Ueber Ptomaine,* i., ii. Berlin, 1885; iii. 1886: gives literature to date. See résumé with references by O. Schultz, *Biol. Centralb.* Bd. vi. (1886–87), Sn. 685, 726, 739. Gautier, *Bull. de l'acad. de med.* Jan. 12, 19, 1886 (largely on the leukomaines). Udránszky u. Baumann, *Zt. f. physiol. Chem.* Bd. xiii. (1889), S. 562. Brieger, Virchow's *Arch.* Bd. cxv. (1889), S. 483. The last contains a most useful list of known ptomaines, with empirical and constitutional formulæ, name of discoverer with date of discovery, sources, action, and characteristic reactions.

Although the substance to which the above name has been given (from πτῶμα, a corpse) are now known to belong chiefly to the class of compounds called amines,[1] so that they provide no chemical sequence to the bodies previously described, their characteristic production during the putrefactive decomposition of animal tissues seems to make this a suitable place for treating of them.

The ptomaines as a group may be said to closely resemble the class of substances long known under the name of alkaloids and obtained from plant tissues. The resemblance is shown not merely in their chemical constitution, but more obviously in their similar solubilities in various fluids, in their general behaviour towards reagents, and in some cases even in their specific reactions, and more especially in their frequently powerful (poisonous) action on the animal organism, the actions of certain ptomaines being almost identical with those of certain vegetable alkaloids. The ptomaines may therefore be regarded as alkaloids of animal origin. The close similarity of the two classes of substances has hence endowed the ptomaines with very considerable interest from a medico-legal point of view, in virtue of the not infrequent use of the vegetable-alkaloids for criminal purposes and the now obvious possibility that the detection of alkaloids in the corpse may afford no reliable information as to the administration of the same during life.[1] They are further of considerable and increasing pathological interest, and that from two points of view. In the first place, as products of the general putrefactive changes which animal tissues undergo, they may account for the severe symptoms and not infrequent death which results from the consumption as food of fish, sausages, and tinned-meats. In the second there appears to be increasing evidence of the formation of special ptomaines by the organisms characteristic of specific diseases, so that the pathological conditions may be due rather to the products formed by the organisms than to the organisms themselves directly, a possibility of no small importance in the light of recent prophylactic research.

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[1] An amine is, strictly speaking, a compound ammonia in which one or more atoms of hydrogen have been replaced by some oxygen-free radical. Several of the ptomaines, however, contain oxygen in their molecule, as do also many of the vegetable alkaloids. The constitution of those ptomaines which contain oxygen has not in most cases as yet been as definitely determined as has that of those which contain none.

While the general reactions of the ptomaines place them, as already stated, side by side with the vegetable alkaloids, their specific reactions and properties exhibit considerable differences both in comparison with each other and with those of the alkaloids.[2] Some are liquid and highly volatile so that they pass off readily during distillation of their aqueous solutions, others are liquid and non-volatile, others again solid and crystalline. They exhibit equally marked differences in their solubilities. Thus neither benzol nor petroleum-ether will extract them from their *acid* aqueous solution. Ether on the other hand dissolves out a few of the ptomaines from an acid solution and a large majority from an alkaline solution. Some are more particularly soluble in chloroform, a few are insoluble in any of these reagents. Amyl-alcohol is the one reagent in which as a class they appear to be almost generally soluble (Brieger). Their behaviour with the usual alkaloidal precipitants (mercuric and platinic chlorides, tannic acid, the double iodides of potassium with mercury and other metals, &c.) is equally varied. They are all precipitated by phospho-molybdic acid, and most of them yield crystalline compounds with a solution of iodine in hydriodic acid. Possessed of an alkaline reaction they further act as powerful reducing agents, many of them converting ferri- into ferrocyanides, the reduction being evidenced by the formation of Prussian blue on the simultaneous addition of ferric chloride. This property is however possessed by many vegetable alkaloids and not by every ptomaine; it cannot therefore be regarded as a specific class-reaction for these substances (Brieger, Gautier). Some of the ptomaines (Toxines) are extraordinarily poisonous, producing effects which are frequently specific, but in many cases similar to those of certain vegetable alkaloids. Others again are quite inert.

The separation of the ptomaines, as of the vegetable alkaloids, involves the application of methods (Stas-Otto's, Brieger's)[3] which admit of no suitably brief description. The principle involved in each consists in preparing a concentrated alcoholic, ethereal, or chloroformic extract of the mother-substance, and from this crystalline compounds of the alkaloids are prepared by the addition of suitable reagents.[1] A further means for their final separation consists in the formation of benzoylated compounds which are insoluble in water.[2]

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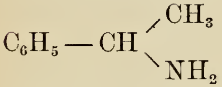
[1] For cases in point see Husemann, *Arch. d. Pharm.* (Reihe 3) Bde. XVI. XVII. XIX. XX. (1882), XXI. (1883), Sn. 169, 327, 187, 270, 401, u. 481.

[2] Otto, *Anleit. zur Ausmittelung d. Gifte*, Aufl. 6, 1884, S. 88 *et seq.*

[3] *Unters. üb. Ptomaine,* II. 1885, S. 52.

Alkaloidal substances, some poisonous, others inert, may also be obtained both from normal but more particularly from pathological urines.[3]

The first distinct evidence that the poisonous properties of certain (septic) fluids might be due to a specific chemical substance rather than necessarily to the action of organisms in those fluids is apparently due to Panum, who seems to have dealt with a septic alkaloid in a very pure form, although he did not definitely characterise it as a chemical substance.[4] This was followed by a series of observations all tending in the same direction, but none of the observers obtained the supposed specifically toxic substances in forms which enabled them to be spoken of as chemical individuals until Nencki in 1876[5] isolated from the products of the pancreatic putrefaction of gelatin a well-crystallised base $C_8H_{11}N$, to which he assigned the constitutional formula  $C_6H_5 - CH\begin{cases} CH_3 \\ NH_2 \end{cases}$ and hence the name isophenyl-ethylamin. Since then the ptomaines have been in most cases fairly definitely and in some cases absolutely characterised as regards their chemical composition and constitution. They belong typically to the class of substances known as amines and are in many cases diamines. Two of the most clearly defined ptomaines are cadaverin and putrescin. These are found in corpses which have undergone putrefactive decomposition, cadaverin appearing in the earlier stages of putrefaction, and putrescin preponderating in the later. The latter is largely present in putrid herrings.[6] The former is identical with pentamethylen-diamine $NH_2(CH_2)_5NH_2$.[7] The latter has been shown to have the constitution of tetramethylen-diamine $NH_2(CH_2)_4NH_2$. They have both recently been obtained as conspicuous constituents of urine from a case of cystinuria, and appear to owe their origin to putrefactive processes occurring in the intestine.[1] They are both somewhat viscid fluids which crystallise at low temperatures, and yield readily crystallisable compounds with acids and salts of gold, platinum, and mercury. Their benzoyl compounds are insoluble in water and hence afford a convenient means for their separation. Cholin and the highly toxic neurin, which really belong to this class, have already been described. (See above pp. 135, 136.)

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[1] For description of these methods see Halliburton, *Chem. Physiol. and Pathol.* 1891, p. 175. Otto, *loc. cit.* S. 103.

[2] Udránszky u. Baumann, *Zt. f. physiol. Chem.* Bd. XIII. (1889), S. 562.

[3] For details and literature see Neubauer u. Vogel, *Anal. d. Harns.* 1890, S 241 *et seq.*

[4] Published originally in Danish in *Bibliothek. f. Läger*, April, 1856, S. 253. Fully abstracted in Schmidt's *Jahrbücher d. ges. Med.* Bd. CI. (1859), S. 213, and republished in Virchow's *Arch.* Bd. LX. (1874), S. 301, with literature up to date.

[5] *Ueb. d. Zersetz. d. Gelatine u. s. w.* Bern, 1876. See later *Jn. f. prakt. Chem.* Bd. XXVI. (1882), S. 47.

[6] Bocklisch, *Ber. d. d. chem. Gesell.* Bd. XVIII. (1885), Sn. 86, 1922; XX. (1887), S. 1441.

[7] Ladenburg, *Ibid.* Bd. XIX. (1886), S. 2585.

Leukomaines. This name has been applied by Gautier[2] to those basic (alkaloidal) substances which occur in *living* tissues and are to be regarded as products of their normal metabolism and thus distinct from ptomaines. They are obtained by extracting finely minced ox-flesh with an extremely dilute aqueous solution of oxalic acid. According to Gautier this extract may contain the following six bases: Xanthokreatinin, $C_5H_{10}N_4O$; Chrysokreatinin, $C_5H_8N_4O$, Amphikreatinin, $C_8H_{19}N_7O_4$, Pseudoxanthin, $C_4H_5N_5O$ and two, as yet unnamed, with the composition $C_{11}H_{24}N_{10}O_5$ and $C_{12}H_{25}N_{11}O_5$ respectively. They probably stand in close relationship to paraxanthin, $C_7H_8N_4O_2$, heteroxanthin, $C_6H_6N_4O_2$, and adenin $C_5H_5N_5$ (see above, p. 181), and it is interesting to note that comparing the formulæ of the leukomaines with each other and with those of kreatinin $C_4H_7N_3O$ and kreatin $C_4H_9N_3O_2$ they are found to differ in several cases by the group CNH.

The leukomaines are regarded by Gautier as feebly toxic alkaloidal products of metabolism from which the organism is normally freed either by their excretion, since they are found in urine (see above), or by destructive oxidation, and it has further been suggested that their abnormal retention in the economy may be the cause of certain obscure pathological conditions.[3]

The Bile-Acids.

1. **Cholalic** (or cholic) **acid.** $C_{24}H_{40}O_5$.

To avoid confusion the term 'cholic' should be in all cases used as synonymous with 'cholalic.' Demarcay, who first described cholalic acid as a product of the decomposition of bile-acids, gave it the name of cholic acid.[4] The name 'cholalic' is perhaps the better, since it indicates the method by which the bile-acids are decomposed during its preparation, viz. by treatment with alkalis. The name 'cholic' was first applied by Gmelin[1] and subsequently by Strecker[2] to the acid which is now always known as glycocholic. The acid now known as taurocholic was originally called 'choleic' by Demarcay, and the same name has been quite recently used to denote an acid ($C_{25}H_{42}O_4$) closely related to cholalic acid (see below).

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[1] Udránszky u. Baumann, *loc. cit.* See also Stadthagen u Brieger, Virchow's *Arch.* Bd. cxv. (1889), S. 490.

[2] *Sur les alcaloides dérivés de la destruction bactérienne ou physiologique des tissus animaux.* Paris, 1886. *Bull. de l'acad. de méd.* Jan. 12, 19, 1886. The name is derived from λεύκωμα, occasionally used to denote white of egg, and hence to indicate their origin from proteids.

[3] Cf. Bouchard, *Compt. Rend.* T. cii. (1886), pp. 669, 727, 1127.

[4] Liebig's *Ann.* Bd. xxvii. (1838), S. 270.

This acid occurs in traces as a product of the decomposition of the bile-acids in the small intestine, in larger quantities in the contents of the large intestine, and in the fæces of man and many animals. In icterus the urine is also stated to frequently contain traces of this acid. Its principal interest lies in its being the starting-point, by its union with glycin or taurin, for the acids which, as sodium salts, exist characteristically in bile (see below).

Owing to the readiness with which ox-bile can be obtained in large quantities, this has been most frequently used for the preparation of cholalic acid, whose properties as usually given hence refer to the acid as obtained from this source. More recent researches have however demonstrated comparatively unimportant but still distinct differences in the composition and properties of the acid as it occurs in the bile-acids of different animals. The description of the acid which here follows refers to that form which is obtained from ox-bile.

Preparation. This depends upon the decomposition of the bile-acids (glycocholic and taurocholic) by means of alkalis at boiling temperature. It is not however necessary to employ the purified acids for this purpose since the raw bile suffices. The bile is boiled for twenty-four hours with as much caustic baryta as it will hold in solution, concentration during this operation being avoided by means of a condenser attached to the mouth of the flask. When the decomposition is complete the fluid is filtered while still hot, and the filtrate concentrated until crystals, consisting of the barium salt of the acid, are copiously formed. These are then purified by recrystallisation from boiling water and decomposed by the addition of hydrochloric acid. The free cholalic acid is finally obtained in a pure form by solution in a small volume of boiling alcohol from which it separates out in the crystalline form on cooling.

As thus prepared the acid possesses the following properties. The crystals obtained from hot alcoholic solutions are usually in the form of large rhombic tetrahedra or octahedra, containing $2\frac{1}{2}$ molecules of water of crystallisation which may be driven off by heating to 100° C. The crystals are but slightly soluble (1 in 750) either in water, even when boiling, or in ether, but readily soluble in alcohol. This acid may also be obtained in an amorphous form by concentrating its solutions to dryness, and is now less insoluble than when crystallised. If the amorphous acid is dissolved in ether it may be separated out by evaporation in four or six-sided prisms which are anhydrous. When the sodium salt of cholalic acid is decomposed under ether by the addition of hydrochloric acid, the acid may be obtained in rhombic plates containing one molecule of water. The alkali and barium salts of cholalic acid are soluble in water and in alcohol, especially when warm, and yield, like the free acid, dextro-rotatory solutions. For solutions of the anhydrous acid $(\alpha)_D = + 50°$. When crystallised with $2\frac{1}{2}$ H_2O, $(\alpha)_D = + 35°$. In alcoholic solutions of the sodium salt $(\alpha)_D = + 31°{\cdot}4$ (Hoppe-Seyler).

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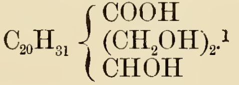
[1] *Die Verdauung nach Versuchen*, 1826.

[2] Liebig's *Ann.* Bd. LXV. (1848), S. 1.

The constitution of cholalic acid is scarcely as yet definitely known, but may be represented by  $C_{20}H_{31}\left\{\begin{array}{l}COOH\\(CH_2OH)_2\\CHOH\end{array}\right.$.[1] It yields with iodine a compound which, like that resulting from the interaction of iodine and starch, possesses a brilliantly blue colour and is specifically distinctive, since it cannot be obtained either from the bile-acids or choleic acid (see below) or the products of the decomposition of cholalic acid.[2]

When cholalic acid is prepared from human bile it exhibits certain differences, more especially as regards the lesser solubilities of its alkali and barium salts, which led to its being regarded as distinct from that obtained from ox-bile, and hence it was called anthropocholalic acid. It appears however that the bulk of the acid is identical with that from ox-bile, the slight difference being due to an admixture with another acid either choleic, as was first supposed, or fellic.[3]

Choleic acid, $C_{25}H_{42}O_4$. Is obtained in small amounts mixed with cholalic acid during the preparation of the latter from ox-bile. It differs from cholalic acid in the solubility of its salts and the products of its oxidational decomposition.[4]

Fellic acid,[5] $C_{23}H_{40}O_4$. Obtained in small amounts from human bile during the preparation of ordinary cholalic acid. It is characterised by the extreme insolubility of its barium and magnesium salts. It also yields a less brilliant Pettenkofer reaction (see below) than does cholalic acid.

The bile-acids of the pig and goose when decomposed yield forms of cholalic acid called respectively hyo-cholalic acid $C_{25}H_{40}O_4$, and chenocholalic $C_{27}H_{44}O_4$.

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[1] Mylius, *Ber. d. d. chem. Gesell.* Bd. xx. (1887), S. 1968.

[2] Mylius, *Ibid.* S. 683 and *Zt. f. physiol. Chem.* Bd. xi. (1887), S. 306. See also Bd. xii. (1888), S. 262.

[3] Schotten, *Zt. f. physiol. Chem.* Bd. x. (1886), S. 175; xi. S. 268.

[4] Latschinoff, *Ber. d. d. chem. Gesell.* Bd. xviii. (1885), S. 3039.

[5] Schotten, *loc. cit.*

2. **Dyslysin.** $C_{24}H_{36}O_3$.

When cholalic acid is heated to 200° C. or boiled for some time in solution with hydrochloric or sulphuric acid it loses two molecules of water and yields a resinous anhydride called dyslysin, from its insolubility in water, alcohol, and alkalis. As resulting from the dehydration of cholalic acid it is found sometimes in small amount in the fæces. It is a non-crystalline substance which is soluble in an excess of ether, also in solutions of cholalic acid or of its salts. By treatment with boiling alkalis it may be reconverted by hydration into cholalic acid.

The various forms of cholalic acid which may be prepared from the bile of different animals each yield a corresponding form of dyslysin.

3. **Glycocholic acid.** $C_{26}H_{43}NO_6$.

This substance was first described by Gmelin (1826), by whom it was then named 'cholic acid.' It is found not in the free state but as a sodium salt, chiefly in ox-bile but also in that of man, mixed in both cases with a much smaller and variable amount of taurocholic acid, also present as a sodium salt. In carnivora it occurs, if at all, in such minute traces only, that it may be said to be absent from the bile of these animals; hence their bile-acid consists entirely of taurocholic acid.[1] In icterus the urine may contain small quantities of glycocholic acid.

Preparation. This may be affected in several ways, using ox-bile as the source; of these the following is as convenient as any (Drechsel).[2] The bile is mixed with washed sand and evaporated on a water-bath until it presents a pulverisable mass. This is then extracted in a flask with strong boiling alcohol and yields a green solution, which is filtered, decolourised with animal charcoal, and concentrated to a sirup. The latter is then dissolved in a minimal quantity of absolute alcohol and precipitated by an excess of ether. The precipitate which consists of glycocholate of soda together with the corresponding salt of any taurocholic acid which is present in the bile, is collected, dissolved in a little water, and acidulated with sulphuric acid in presence of some ether as long as any precipitate is formed. By this means the acids are separated from their sodium salts, and on standing a crystalline mass of glycocholic acid is obtained, practically free from taurocholic acid, which, since it is, unlike the glycocholic, extremely soluble in cold water, remains in solution in the mother liquor. The crystals may be purified by recrystallisation from

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[1] For earlier references to the bile-acids of various animals see Bayer, *Zt. f. physiol. Chem.* Bd. III. (1879), S. 293.

[2] *Anleit. z. Darstell. physiol.-chem. Präparate*, 1889, S. 33.

hot water in which they are soluble, separating out again as their solution cools.[1]

The acid crystallises in fine glistening needles, which require about 300 parts of cold but only 120 of hot water for their solution. They are also very soluble in alcohol, but practically insoluble in ether. The salts of this acid, more especially those with the alkalis, are extremely soluble even in cold water, also in alcohol, but very slightly so if at all in ether. Both the free acid and its salts are dextro-rotatory: for the former, in alcoholic solutions, $(\alpha)_D = +29{\cdot}0°$, for the latter $(\alpha)_D = +25{\cdot}7°$ (Hoppe-Seyler). Glycocholic acid is a compound of cholalic acid and glycin (glycocoll) or amido-acetic acid. When boiled with hydrolysing agents such as dilute acids or alkalis it takes up one molecule of water and is resolved into its components:—

$$C_{26}H_{43}NO_6 + H_2O = \overset{\text{Glycin.}}{CH_2\,(NH_2)\,COOH} + \overset{\text{Cholalic acid.}}{C_{24}H_{40}O_5}.$$

It is thus analogous in constitution to hippuric acid, in which glycin is similarly united to benzoic acid.

If dissolved in concentrated sulphuric acid and then warmed, glycocholic acid by the removal of one molecule of water yields glycocholonic acid, $C_{26}H_{41}NO_5$. The barium salt of this last acid is insoluble in water, which fact is of importance, since cholonic acid possesses nearly the same specific rotatory power as glycocholic acid.

4. **Taurocholic acid.** $C_{26}H_{45}NSO_7$.

This acid is found to some extent in ox-bile, and is more plentifully present in that of man. The bile of the dog contains taurocholic acid alone, unmixed with glycocholic.

Preparation. The method described above suffices to obtain glycocholic acid free from taurocholic. On the other hand it is not by any means easy to obtain the latter free from the former, for taurocholic acid is extremely soluble in water (its crystals are deliquescent) and this solution can readily dissolve the much less readily soluble glycocholic acid. Hence the mother liquor from the glycocholic acid crystals contains not merely the taurocholic acid but some of the former acid also. This difficulty is avoided by employing as a source for its preparation dog-bile in which there is no glycocholic acid. The bile is treated as already described down to the stage at which the taurocholate of soda is precipitated from its alcoholic solution by an excess of ether. The precipitate is now dissolved in water and the acid precipitated as a lead salt by the addition of ammonia and *basic* lead-acetate.

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[1] For details of other methods some special work should be consulted, such as Hoppe-Seyler's *Handbuch*. See also Maly in Hermann's *Handbuch d. Physiol.* Bd. v. Th. 2, S. 130. Cf. Mylius, *Zt. f. physiol. Chem.* Bd. xi. (1887), S. 231.

This is next washed, suspended in alcohol, and decomposed by sulphuretted hydrogen. After removal of the sulphide of lead by filtration the alcoholic filtrate is concentrated and the taurocholic acid precipitated by an excess of ether. This yields a sirupy mass which may become partly crystalline on standing: the crystals at once deliquesce on exposure to the air.[1] As dog-bile is not readily obtainable in large quantity at any one time, it may be desirable sometimes to obtain taurocholic acid from the mother liquor left in the preparation of glycocholic acid. The separation is effected by the addition of a *little* ammonia and *normal* lead acetate. This precipitates both glycocholic and cholalic acid, but not taurocholic. After the removal of this precipitate the taurocholic acid is prepared as already described by the addition of basic lead acetate to the filtrate.

This acid, as already stated, is extremely soluble in water and in alcohol, but not in ether; so also are its salts with the exception of the one formed on the addition of basic lead acetate in presence of ammonia, which is insoluble in water and in alcohol. The acid and its salts are dextro-rotatory; for the sodium salt in alcoholic solution $(\alpha)_D = +24{\cdot}5°$. If dissolved in water the rotatory power is less, and in this respect it resembles glycocholic acid.

When hydrolised it readily takes up a molecule of water and is decomposed into taurin and cholalic acid:—

$$C_{26}H_{45}NSO_7 + H_2O = \overset{\text{Taurin}}{NH_2 . CH_2 . CH_2 . SO_2OH} + \overset{\text{Cholalic acid.}}{C_{24}H_{40}O_5}.$$

This decomposition may, as in the case of glycocholic acid, be brought about by the action of dilute acids or alkalis, but even mere boiling of an aqueous solution of the acid also suffices, a fact which demonstrates how unstable a substance it is, both absolutely and as compared with glycocholic acid. Taurocholic acid has not as yet been observed in the urine in icterus, but since cholalic acid does occur together with glycocholic acid, it is probable that the non-appearance of taurocholic acid is due to its decomposition before excretion as a result of its instability.

Taurocholic acid possesses a remarkable power of effecting the complete precipitation of ordinary proteids from their solutions, whereas peptones if present at the same time remain unprecipitated. This is possibly of some not inconsiderable importance in connection with the changes which proteids undergo in the small intestine, since it leads to the retention of the peptones in a state of solution and hence facilitates their absorption, while the less completely altered proteids are precipitated and thus further exposed to the action of the digestive enzymes.[1] It is also possessed of powerful antiseptic properties.[2]

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[1] Parke, Hoppe-Seyler's *Med.-chem. Unters.* Hft. 1. (1866), S. 160.

The acids obtained from the bile of different animals differ slightly in properties and composition, dependently, as already stated, upon the differences between the several forms of cholalic acid with which either the glycin or taurin is respectively united.

Pettenkofer's reaction for bile acids.[3]

The following is the more usual method of obtaining the reaction. Bile, which may be very considerably diluted, or a dilute solution of bile-salts or acids is mixed in a porcelain dish with a few drops of a 10 p. c. solution of cane-sugar. Concentrated sulphuric acid is now added to the mixture with constant stirring to an extent not exceeding $\frac{2}{3}$ of its volume, the addition of the acid being so regulated that the temperature of the mixture is not allowed to rise above 70° C. Hereupon a brilliant cherry-red colour makes its appearance and rapidly assumes a magnificent purple tint. On standing for some time the colour becomes darker and of a more distinctly blue tint. The reaction may also be obtained by the addition of first the acid and then the sugar solution. The success of the test depends on the careful avoidance of any excessive rise of temperature during the addition of the sulphuric acid and more especially of any excess of sugar which by being charred by the acid gives a brown colouration and masks the typical purple.[4] The purple solution if diluted with alcohol (not with water, which destroys the colour) shows with a spectroscope a characteristic absorption spectrum consisting of two absorption bands, one between D and E abutting on E, and a second adjoining the F line. In the earlier stages of the reaction a third narrow band near D makes its appearance but disappears later on.[5]

Pettenkofer's reaction depends upon the presence in all bile-acids of their cholalic acid constituent. On the first addition of sulphuric acid, if the solution be at all concentrated, a white precipitate may often be observed consisting of cholalic acid; this is dissolved on the further addition of acid, after which the characteristic colour makes its appearance. It has also recently been shown that the reaction depends upon the formation of fur-

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[1] Maly u. Emich, *Monatshefte f. Chem.* Bd. IV. (1883), S. 89. See also Hammarsten, Pflüger's *Arch.* Bd. III. (1870), S. 53. On the similar behaviour of taurocholic acid to gelatin and its peptones see Emich, *Monatshefte f. Chem.* Bd. VI. (1885), S. 95.

[2] Maly u. Emich, *loc. cit.* See also Lindberger (Swedish). See Abstr. in Maly's *Jahresb.* 1884, S. 334.

[3] Pettenkofer, *Annal. d. Chem. u. Pharm.* Bd. LII. (1844), S. 90.

[4] To avoid this, Drechsel recommends the employment of phosphoric acid (5 of glacial acid to 1 of water) instead of sulphuric acid, *Jn. f. prakt. Chem.* Bd. XXIV. (1881), S. 44; XXVII. (1883), S. 424. In this case the solution must be heated by immersion in boiling water.

[5] Schenk. See ref. in Maly's *Jahresb.* 1872, S. 232. Udránszky, *Zt. f. physiol. Chem.* Bd. XII. (1888), S. 372. Mac Munn, *Clin. chem. of urine*, 1889, p. 174.

furol[1] by the action of the sulphuric acid upon the sugar, the colour arising from the interaction of furfurol with cholalic acid.[2]

It is important to remember that an extended series of substances other than cholalic acid and the bile-acids (pigments and other substances which are charred by sulphuric acid) either interfere with the brilliancy of the reaction or else themselves yield a purple colour which closely resembles that due to the bile-acids. Among the latter those of chief importance are proteids, amyl-alcohol, oleic acid, the higher fatty acids, and cholesterin.[3] A further element of uncertainty is introduced by the fact that if the suspected solution be extremely dilute no characteristic colour is obtained although bile-acids may be present. All the above militate against the detection of bile-acids in fluids such as urine, in which their determination is a matter of not infrequent importance. The application of Pettenkofer's reaction in its original form has hence been modified in details by many observers with a view to rendering it more decisive and delicate. The decisiveness of the reaction is ensured by careful spectroscopic examination of the absorption spectrum of the coloured solution, since the colours produced by the majority of those substances which yield a reaction resembling that produced by cholalic acid, show no absorption bands in their spectra. Some few however do exhibit absorption bands which fortunately occupy a different position in the spectrum from those shown by cholalic acid (Udránszky). If the suspected solution is extremely dilute it may frequently be made to yield Pettenkofer's reaction directly by a previous concentration on the water-bath. A further modification which is applicable to dilute solutions is the following. A little cane-sugar is dissolved in the solution and a strip of filter-paper dipped into it and then air-dried. When dry one drop of concentrated sulphuric acid is applied to the paper with a glass rod. If bile-salts are present (even to the extent of ·03 p. c.) a distinct violet stain may be observed on the paper after standing for a quarter of a minute: the stain is most easily seen by transmitted light.[4] Instead of sugar an aqueous (0·1 p. c.) solution of furfurol may be used to great advantage as follows. One drop of this solution is added to 1 c. c. of the suspected solution, either aqueous or alcoholic, in a test tube. To the above is then added 1 c. c. of concentrated sulphuric acid and the mixture is cooled under water so that its temperature does not exceed 50° — 60° C. To detect bile-acids in urine with absolute certainty it is essential to separate them from this excretion before applying Pettenkofer's test. This is effected either by precipitation with basic lead acetate or extraction with alcohol or chloroform.[1]

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[1] Also known as furfuraldehyde $C_4H_3O.COH$, the aldehyde of pyromucic acid $C_4H_3O.COOH$.

[2] Mylius, *Zt. f. physiol. Chem.* Bd. XI. (1887), S. 492.

[3] For a complete list of these see Udránszky, *loc. cit.* S. 358.

[4] Strassburg, Pflüger's *Arch.* Bd. IV. (1871), S. 461.

THE COLOURING MATTERS AND PIGMENTS OF THE ANIMAL BODY.

HÆMOGLOBIN AND ITS DERIVATIVES.

1. **Hæmoglobin.**[2] This is the well-known constituent of the red corpuscles to which the dark colour of the blood from an asphyxiated animal is due. It is also present to a less and somewhat variable amount in ordinary venous blood, in presence of correspondingly variable amounts of the compound which it forms with oxygen, namely oxy-hæmoglobin. In normal arterial blood it is probably present in mere traces, if at all, since here its affinities for oxygen are completely satisfied to form oxy-hæmoglobin. Hæmoglobin is chiefly of interest as an oxygen-carrier or respiratory pigment, in virtue of the ease with which it absorbs and unites in loose combination with oxygen when merely exposed to this gas, and again gives it up when brought into relationship with the oxygen-free tissues of the body. The conditions and phenomena of this fixation and liberation of oxygen by hæmoglobin have been very fully investigated; the fundamentally important facts in connection with it have already been stated in some detail in an earlier part of this work (§ 343 et seq.), so that it is now only necessary to add some further details of hæmoglobin of a more purely chemical character.

Owing to the ease and avidity with which hæmoglobin unites with oxygen to form the distinct and stable compound known as oxy-hæmoglobin, its investigation is attended with considerable experimental difficulties; hence our knowledge of it as a chemical substance is on the whole less complete than is that of oxy-hæmoglobin. Hæmoglobin may be obtained in a crystalline form,[3] but with some considerable difficulty owing to its extreme solubility in water. The crystals may be prepared by sealing up a concentrated aqueous solution of oxy-hæmoglobin in glass tubes from which, if necessary, all remaining air is displaced by hydrogen: on prolonged standing all the oxygen disappears during the putrefactive reduction which ensues, and finally, more readily on exposure to a low temperature, crystals of hæmoglobin make their appearance in the fluid.[1] A similar production and formation of crystals is frequently observed when crystals of oxy-hæmoglobin are sealed up with Canada balsam under a cover-slip and kept for some time.[2] The form of the crystals obtained from the blood of different animals has not yet been fully investigated. They exhibit to a marked degree the phenomena of pleochroism, being apparently trichromatic.[3]

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[1] For details see Hoppe-Seyler, *Hdbch. d. phys.-path. Chem. Anal.* 1883, S. 399, and Neubauer u. Vogel, *Analyse d. Harns*, 1890, S. 146.

[2] The single name hæmoglobin is used here to denote what is more frequently and usually called 'reduced' hæmoglobin, as distinct from oxy-hæmoglobin. The adoption of the name as here used is both simpler and more logical.

[3] First described by Kühne, Virchow's *Arch.* Bd. xxxiv. (1865), S. 423.

Pleochroism is that property possessed by many crystals of appearing to differ more or less in colour, in accordance with the direction from which they are viewed by transmitted light. The phenomena are usually investigated by means of a single Nicol prism. For further details consult some special work on mineralogy or the article on this subject in the "Encyclopædia Britannica," Vol. XVI. p. 375.

As ordinarily seen the crystals of hæmoglobin have a dark red appearance, unlike the bright scarlet of oxy-hæmoglobin, with a strong purple or bluish tint. They are extremely soluble in water, much more so than the crystals of oxy-hæmoglobin. The optical properties of solutions of hæmoglobin have already been sufficiently described (§ 346, and see below Fig. 36, No. 5). One of the most remarkable properties of hæmoglobin is its power of uniting directly with any one of several gases, such as oxygen, carbon monoxide, nitric oxide and, as recent research has shown, possibly carbon dioxide; the compounds which are thus formed have in the case of the first three gases a definite and constant composition, crystallising more or less readily in characteristic forms and showing in aqueous solutions absorption spectra which are constant and characteristic for each. (See below.)

The chemical composition of hæmoglobin does not as yet admit of being represented by any definite formula, and indeed its percentage composition has not been determined by direct analysis. It must be inferred from a knowledge of the probable composition of the more stable and easily crystallisable oxy-hæmoglobin and of the quantitative relationships which hold good between hæmoglobin and oxygen during its conversion into oxy-hæmoglobin. As will be seen later on, analysis of purified crystals of oxy-hæmoglobin shows that these probably differ in composition as prepared from the blood of different animals, and the same statement therefore probably holds good for hæmoglobin. When decomposed in the absence of oxygen (air), as for instance by the action of organic acids, more dilute mineral acids, or best of all by caustic alkalis, it yields a proteid, of which but little is known (see p. 32), and a coloured substance called by Hoppe-Seyler hæmochromogen. The latter on exposure to air absorbs oxygen and becomes ordinary hæmatin; it is in fact the substance usually spoken of as reduced hæmatin. (See below.)

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[1] Hüfner, *Zt. f. physiol. Chem.* Bd. IV. (1880), S. 382. Cf. Nencki u. Sieber, *Ber. d. d. chem. Gesell.* Bd. XIX. (1886), Sn. 129, 410.

[2] A. Ewald, *Zt. f. Biol.* Bd. XXII. (1886), S. 459.

[3] A. Ewald, *loc. cit.*

2. **Oxy-hæmoglobin.** When hæmoglobin is exposed to the air it rapidly unites, molecule for molecule, with oxygen, thus becoming oxy-hæmoglobin, the characteristic constituent of the red-corpuscles to which the scarlet colour of arterial blood is due.[1] It may be readily set free from the corpuscles by the addition to defibrinated blood of such fluids as alcohol, ether, chloroform, water, and solutions of bile-salts, or by repeatedly freezing and thawing the blood; when thus set free it passes into solution in the adjacent serum. From this solution it may be obtained as crystals with more or less readiness, dependently upon the kind of animal whose blood is used for its preparation (see § 344), the difference being due, partly at least, to the varying solubility of the several hæmoglobins.

To obtain rapidly a microscopic preparation of oxy-hæmoglobin crystals it suffices to take a drop of the blood of some animal whose hæmoglobin crystallises readily (rat, guinea-pig, or dog), to mix a drop of it on a slide with a minute drop of water, and allow the mixture to evaporate until a ring of dried substance is formed at the periphery. If it be now covered with a cover-slip, crystals usually form in a short time, especially if it be kept cooled. For the preparation of oxy-hæmoglobin crystals on a large scale many methods, the same in general principles but differing somewhat in detail, have been proposed, the difficulty of the preparation varying considerably according to the kind of blood used.[2] For laboratory purposes large quantities of crystallised oxy-hæmoglobin may be very readily obtained from dog's blood as follows (Kühne). The blood is defibrinated and strained through fine muslin: it is then placed in a flask and ether is added with frequent shaking until the blood is just 'laky,' i. e. transparent. The flask is now surrounded by a freezing mixture of ice and salt and in a short time its contents usually become almost pasty from the mass of crystals which form in it. These are then centrifugalised off, dissolved in a *minimal* amount of water, filtered, cooled to 0°, and after the addition of one quarter of its bulk of cooled alcohol again immersed in a freezing mixture. The second crop of crystals thus obtained may be again recrystallised as already described. The crystals are finally washed with water at 0° containing 25 p. c. of alcohol, and may be dried in vacuo over sulphuric acid at 0°, and are now fairly stable.

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[1] Hæmoglobin is united to corpuscles in the blood of all vertebrates, with two exceptions. In invertebrate blood it is usually found in solution in the plasma, but there are a few (eight) exceptions to this rule. For details and literature see Halliburton, *Chem. Physiol. and Pathol.* 1891, pp. 267, 316.

[2] For fuller details see Gamgee, *Physiol. Chemistry*, Vol. I. 1880, p. 85. See later Otto, *Zt. f. physiol. Chem.* Bd. VII. (1882), S. 57. Zinoffsky, *Ibid.* Bd. X. (1885), S. 18. Hüfner, *Beitr. z. Physiol. Festschr. f. C. Ludwig*, 1887, S. 74. Mayet, *Compt. Rend.* T. 109 (1890), p. 156.

The crystals obtained from the hæmoglobin of various animals differ in their crystalline form. The following figure shows some of the most typical forms.[1]

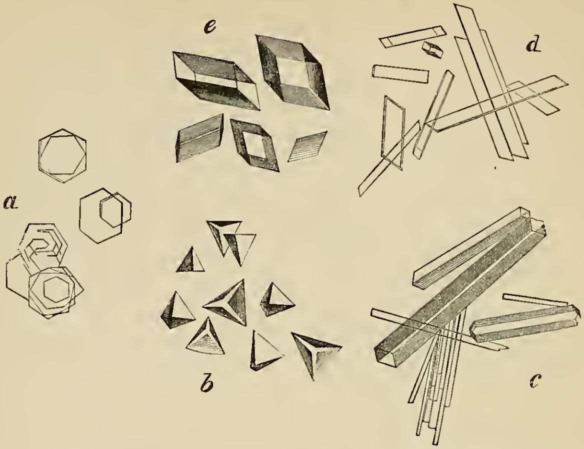

FIG. 35. CRYSTALS OF OXY-HÆMOGLOBIN. (After Funke.)
a. Squirrel, *b.* Guinea-pig, *c.* Cat, or Dog, *d.* Man, *e.* Hamster.

Apart from these differences in crystalline form the oxy-hæmoglobin of different animals varies in its solubility, in the amount of water of crystallisation with which its crystals are united, and also apparently in its percentage composition. The crystals are pleochroic but to a less extent than are those of hæmoglobin.[2] As against these differences it is important to notice that the close relationship of the various forms of oxy-hæmoglobin, from whatever blood they may be obtained, is shown by the fact that the spectroscopic properties are in all cases identical, as also are the products of decomposition and the compounds formed with gases. Numerous analyses of oxy-hæmoglobin have been made,[3] but these while they tell us at most that it consists of oxygen, hydrogen, nitrogen, and carbon together with iron as a characteristic constituent and some sulphur, and seem to indicate that it differs in composition as obtained from different animals, do not as yet enable us to assign with any certainty a definite formula to its composition. It is however certain that its molecular weight is enormously great (13,000 — 14,000).[4]

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[1] For a discussion of the various crystalline forms of oxy-hæmoglobin see Halliburton, *Chem. Physiol. and Pathol.* 1891, p. 270.

[2] A. Ewald, *loc. cit.* (sub hæmoglobin).

[3] See Hammarsten's *Lehrb. d. physiol. Chem.* 1891, S. 57; or Halliburton's *Text-book of Chem. Physiol. Pathol.* 1891, p. 286.

[4] Marshall, *Zt. f. physiol. Chem.* Bd. VII. (1882), S. 81. Külz, *Ibid.* S. 384. Cf. Zinoffsky, *Ibid.* Bd. X. (1886), S. 16, and see Hüfner, *loc. cit.*

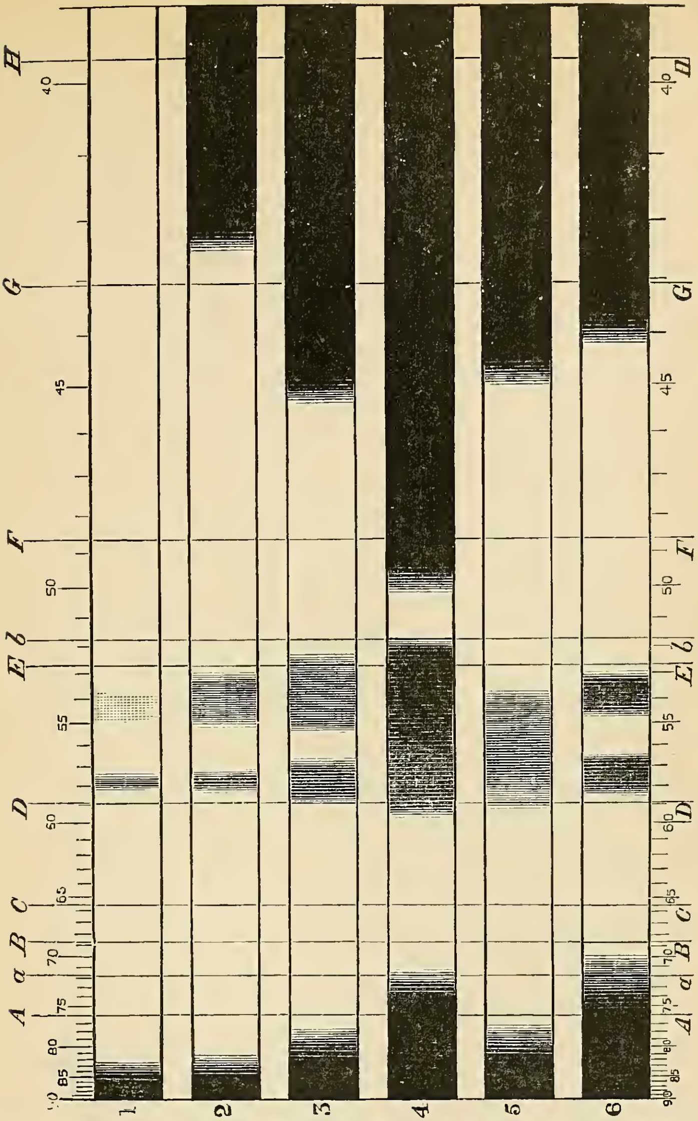

Fig. 36. (After Preyer and Gamgee.) The Spectra of Oxy-hæmoglobin in different grades of concentration, of (reduced) Hæmoglobin, and of Carbon-Monoxide-Hæmoglobin.

1 to 4. Solution of Oxy-hæmoglobin containing (1) less than ·01 p.c., (2) ·09 p.c., (3) ·37 p.c., (4) ·8 p.c.
5. " " (reduced) Hæmoglobin containing about ·2 p.c.
6. " " carbon-monoxide Hæmoglobin.

In each of the six cases the layer brought before the spectroscope was 1 cm. in thickness. The letters (*A*, *a*, &c.) indicate Frauenhofer's lines, and the figures wave-lengths expressed in 100,000th of a millimeter.

The spectroscopic appearances of solutions of oxy-hæmoglobin have been already sufficiently described and figured (§ 345). (For convenience of reference Fig. 75 is reproduced here.) When its solutions are heated or it is treated either in solution or as a solid with acids or alkalis, it may be readily decomposed, yielding a proteid as in the case of hæmoglobin and a coloured residue, viz. hæmatin. (See below.) The oxygen which is loosely combined with hæmoglobin in the formation of oxy-hæmoglobin may be readily removed by several means of which the following are those most usually employed.

(i) The solution is warmed to 40° and the gas driven off by exposure to the vacuum of a mercurial pump. (ii) A current of some neutral gas such as hydrogen or nitrogen is passed through the solution. (iii) The solution is treated with a few drops of some reducing agent such as Stokes' fluid.[1] This is prepared by adding tartaric or citric acid to a solution of ferrous sulphate, and then ammonia until it is strongly alkaline. This reagent does not keep and must be freshly prepared each time it is required. Instead of Stokes' fluid, ammonium sulphide may be used, but in this case some slight manipulation is frequently required to ensure reduction. A few drops of the sulphide are added to the solution, which is then gently warmed: if on examination with the spectroscope it is found that the reduction has not taken place, as shown by the persistence of the two bands of oxy-hæmoglobin, a little more of the sulphide may be added and the mixture again carefully warmed.

The amount of oxygen, removable by the means just described, with which one gram of hæmoglobin (from dog's blood) can unite is usually stated as being 1·59 c.c. at 0° and 760 mm. Hg. this constant being taken as independent of the concentration of the solutions employed.[2] Quite recently some doubt has been cast on the quantity being thus constant; and it has been stated that several modifications of hæmoglobin exist which, while they cannot be discriminated by their purely chemical characteristics, exhibit a marked difference as to the amount of oxygen with which the same quantity of each can unite under similar external conditions; the results thus obtained are stated to hold good for the compound of oxygen with hæmoglobin as it exists in the red blood-corpuscles of the dog,[3] and further for the hæmoglobin of guinea-pigs and geese.[4] Further investigation must decide the interesting questions raised by the above statements.

There appears to be a consensus of opinion that hæmoglobin, and more particularly oxy-hæmoglobin, possesses to a slight degree the properties of an acid. This view appears to be based on the following facts. Oxy-hæmoglobin is extraordinarily soluble in alkalis and in this solution appears to be more stable than ordinarily. It is further stated that it has a feeble power of facilitating the evolution of carbon-dioxide from dilute solutions of sodium carbonate.[1] It is hence often supposed that in the red blood-corpuscles the hæmoglobin is united to the alkalis of which their stroma partially consists. If the above views are correct they may assist in explaining to some slight extent the difficulties in understanding the causes of the exit of carbon-dioxide from venous blood during its passage through the lungs. (See § 357.) But the possibility here indicated must be received with the greatest caution; for it has been shown that although a dilute alkaline solution of oxy-hæmoglobin when exposed to a low partial pressure of carbon-dioxide absorbs less of this gas than suffices to convert the alkali into bicarbonate, thus acting like an acid, at higher partial pressures it absorbs more than can be accounted for by the change of the alkali into bicarbonate. In the latter case the hæmoglobin seems to act like a feeble base.[2] It is interesting here to notice that if the immediately preceding statements hold good, the hæmoglobin must possess increasingly acid properties in proportion as the carbon-dioxide begins to be evolved from the blood, and might thus further that exit. The power apparently possessed by hæmoglobin of itself uniting directly with carbon-dioxide will be referred to again later on.

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[1] *Proc. Roy. Soc.* June, 1864. *Phil. Mag.* November, 1864.

[2] Hüfner, *Zt. f. physiol. Chem.* Bd. I. (1878), Sn. 317, 386. See also *Jn. f. prakt. Chem.* Bd. XXII. (1880), S. 362.

[3] Bohr u. Torup, *Skandinav. Arch. f. Physiol.* Bd. III. Hft. 1, 2 (1891), S. 69. Bohr, *Ibid.* Sn. 76, 101.

[4] Jolin, *Arch. f. Physiol.* Jahrg. 1889, S. 265.

3. **Carbon-monoxide hæmoglobin.** When a current of carbon-monoxide is passed through a solution of oxy-hæmoglobin the oxygen is driven off and its place taken by the first-named gas. The compound thus formed results, like oxy-hæmoglobin, from the union of one molecule of the gas with one of hæmoglobin. It further resembles oxy-hæmoglobin in being readily crystallisable[3] in forms isomorphous with those of the former, but the crystals are on the whole less soluble, brighter coloured and more stable than are those of oxy-hæmoglobin.[4] They are distinctly dichromatic (see p. 216). The compound of carbon-monoxide with hæmoglobin is much more stable than is oxy-hæmoglobin, so that the gas is not again expelled by the action of oxygen, a fact which fully explains the fatal result of breathing carbon-monoxide. Finally the spectrum of carbon-monoxide hæmoglobin while very similar at first sight to that of oxy-hæmoglobin, differs distinctly

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[1] Preyer, *Die Blutkrystalle*, 1871, S. 70.

[2] Setschenow, *Mém. de l'acad. de St. Petersb.* T. XXVI. 1879, confirmed by Zuntz, Hermann's *Hdbch. d. Physiol.* Bd. IV. Th. 2 (1882), S. 76.

[3] For preparation in quantity see Külz, *Zt. f. physiol. Chem.* Bd. VII. (1882), S. 385.

[4] Carbon-monoxide hæmoglobin is unaffected by either putrefactive changes or the action of pancreatic juice. Hoppe-Seyler, *Ibid.* Bd. I. (1877), S. 131.

from it in the position of its two absorption bands (see Fig. 36, No. 6). The spectrum of this compound undergoes no change by the action of any of the reducing agents described on p. 220: this affords a further characteristic means of discriminating between the compounds of carbon-monoxide and oxygen with hæmoglobin. Since the determination of this compound in blood is frequently of considerable importance in medical jurisprudence, many tests for its presence have been devised additionally to the evidence afforded by the spectroscope. One of the oldest and best is due to Hoppe-Seyler.[1] It consists in adding to the suspected blood twice its volume of caustic soda of sp. gr. 1·3. If carbon-monoxide hæmoglobin is present it yields a brilliant red precipitate, differing entirely in appearance from the brownish-green mass observed if oxy-hæmoglobin is present. For further tests consult the literature quoted below.[2]

4. **Nitric oxide hæmoglobin.** If a current of nitric oxide be passed through a solution of carbon-monoxide hæmoglobin, the carbon-monoxide is displaced by the former gas.[3] The compound thus obtained is still more stable than is carbon-monoxide hæmoglobin. It may be crystallised and in solution exhibits two absorption bands very similar to those of oxy-hæmoglobin but slightly nearer the red end of the spectrum; these bands are not affected by reducing agents. If prepared by passing the gas through ordinary blood, the latter should first be freed from oxygen by a current of hydrogen and care must be taken to neutralise the nitrous acid formed during the process.

5. **Carbon-dioxide hæmoglobin.** The possible union of carbon-dioxide with hæmoglobin has already been referred to (p. 221), and more recent researches have thrown further, though still far from complete light upon this possibility. There appears to be no doubt that a solution of hæmoglobin takes up a much larger volume of carbon-dioxide than can be accounted for as the result of a merely physical absorption. Thus in one set of experiments it was found[4] that 1 gr. of hæmoglobin could unite with 2·366 c.c. of the gas at a temperature of 18·4° and partial pressure of 31·98 mm. of Hg, the latter being a mean average partial pressure of carbon-dioxide in venous blood according to the older

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[1] Virchow's *Arch.* Bd. XIII. (1858), S. 104. For a recent modification of this test see E. Salkowski, *Zt. f. physiol. Chem.* Bd. XII. (1888), S. 227.

[2] Jäderholm (Swedish), Abst. in Maly's *Jahresb.* 1874, S. 102. Weyl u. von Anrep, *Arch. f. Physiol.* Jahrg. 1880, S. 227. Zaleski, *Zt. f. physiol. Chem.* Bd. IX. (1885), S. 225. Kunkel, *Sitzb. d. Würzb. physik.-med. Gesell.* 1888, Sitz. 9. Katayama, Virchow's *Arch.* Bd. CXIV. (1889), S. 53. Welzel, *Verhandl. d. physik.-med. Gesell.* Würzb. (N. F.) Bd. XXIII. (1889), S. 3.

[3] L. Hermann, *Arch. f. Anat. u. Physiol.* Jahrg. 1865, S. 409.

[4] Bohr, see *Beiträge z. Physiol. Ludwig, gewidmet*, 1887, S. 164. *Centralb. f. Physiol.* Bd. IV. (1890), S. 253. *Skandinav. Arch. f. Physiol.* Bd. III. Hf. 1, 2 (1891), S. 47. See also Jolin, *Arch. f. Physiol.* Jahrg. 1889, Sn. 277, 285.

established data,[1] while that in arterial blood is 21·28 mm.[2] It is further stated that the stronger solutions of hæmoglobin absorb relatively less carbon-dioxide than the weaker, and that, as in the case of oxy-hæmoglobin (see p. 221) various modifications of hæmoglobin exist possessing different powers of uniting with this gas. On comparing the amounts of carbon-dioxide and of oxygen or CO or NO which may unite with a given weight of hæmoglobin it is at once evident that the mode of union of the former gas must be different from that of the latter three, with which, as already stated, hæmoglobin unites molecule for molecule. This difference in behaviour is very probably due to the decomposition which hæmoglobin undergoes when a current of carbon-dioxide is passed through it,[3] and indeed it is hence probable that the so-called carbon-dioxide hæmoglobin is rather a compound of the gas with a coloured product of the decomposition of hæmoglobin, viz. hæmochromogen, which has been shown by Hoppe-Seyler to unite with carbon-monoxide (see below). The compound, whatever be its true nature, is stated to exhibit a one-banded absorption spectrum closely similar to that of hæmoglobin, but the centre of the band lies slightly more towards the violet end of the spectrum.[4] Bohr states that the absorption of carbon-dioxide is independent of the simultaneous presence of oxygen.[5]

The accurate quantitative determination of the amount of hæmoglobin in any given solution is a matter of extreme importance, not merely in connection with several of the statements contained in the preceding description of hæmoglobin and its compounds with gases, but also in many investigations which turn on the varying amounts of this substance under different experimental conditions, and further for clinical purposes. It may therefore not be out of place to describe briefly the principles on which the determinations are based, referring the reader to special works for the details of the respective processes.

The methods employed fall under two categories: chemical and physical.

1. **Chemical.** *a.* The amount of iron present in 100 parts of hæmoglobin has been frequently determined for the blood of various animals. It may be stated to be about ·43—·45 p.c. Hence if a solution of this substance be evaporated to dryness and the residue incinerated, the amount of hæmoglobin may be inferred from the

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[1] See Wolffberg, Pflüger's *Arch.* Bd. VI. (1872), S. 23. Strassburg, *Ibid.* S. 65 Nussbaum, *Ibid.* Bd. VII. (1873), S. 296.

[2] But cf. Bohr, *Centralb. f. Physiol.* Bd. I. (1887), S. 293, II. (1888), S. 437, who makes it much less. According to this observer the partial pressure of CO_2 in blood is less than that of expired air, and that of oxygen is greater. If this should prove to be the case on further investigation, it would appear that the gaseous interchange which takes place in the lungs cannot be the result of a purely diffusive process, as it is now held to be (§ 354—357).

[3] Torup (Swedish). See Abst. in Maly's *Jahresb.* 1887, S. 115.

[4] Torup, *loc. cit.* and see also "Ueber die Kohlensäurebindung des Blutes," Kopenhagen, 1887.

[5] *Skandinav. Arch. f. Physiol.* Bd. III. (1891), S. 62.

amount of iron, existing as oxide of iron, in this residue.[1] *b.* Since the volume of oxygen which unites with a given quantity of hæmoglobin is known with considerable accuracy (but see above, p. 221), the amount of this substance may be determined by saturating its solution with oxygen and then estimating the volume of the gas which is united to the hæmoglobin. The determination is made either by extracting the oxygen with a mercurial pump or displacing it by carbon-monoxide, or estimating it in the solution by a volumetric process with sodium sulphite and indigo.[2] These methods are inferior to the following.

2. **Physical**. These may be again divided into two: colorimetric and spectrophotometric.

(i) *Colorimetric method.* The principle of this method may be briefly stated as follows. A standard solution of hæmoglobin is prepared from pure crystals of the substance. The tint of the solution in which the hæmoglobin is to be determined is then compared with that of the standard solution: if it is not the same when examined under the same conditions, it must be equalised by either of the methods to be next described; and from the operations necessary to produce equality of tint the *relative* concentrations of the two solutions may be inferred, and hence the *absolute* concentration of the unknown solution. The methods more usually employed consist either in diluting one or the other of the solutions until their tint is the same when examined in layers of *equal* thickness (Hoppe-Seyler),[3] or else in determining the different thicknesses of the fluid layer of each which exhibits the same tint (Duboscq). Since in the latter case the concentrations of the two solutions are inversely proportional to the thicknesses of their layers when their tint is the same, the amount of hæmoglobin in the solution of unknown strength can be at once inferred.[4] For clinical purposes Gower's hæmoglobinometer is perhaps most frequently employed. In this instrument a measured volume of blood is diluted till it has the same tint as that of a standard mass of gelatin coloured with carmine and picrocarmine.[5] There are, however, many other forms of colorimeter designed for clinical use.

(ii) *Spectrophotometric method.* All coloured substances in solution possess the power of absorbing light. With a given thickness of a given substance the amount of light transmitted by the solution bears to the incident light a ratio which, while it varies for different parts of the spectrum, is constant for any one portion, and is therefore characteristic of each substance. Hence if the absolute absorb-

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[1] Pelouze, *Compt. Rend.* T. I. (1865), p. 880.

[2] Gréhant, *Compt. Rend.* T. LXXV. (1872), p. 495. Quinquand, *Ibid.* T. LXXVI. p. 1489. Schützenberger et Risler, *Ibid.* p. 440.

[3] *Hdbch. d. physiol. pathol.-chem. Anal.* Aufl. 5 (1883), S. 435

[4] For a description of Duboscq's and other apparatus see G. u. H. Krüss, *Kolorimetrie u. quant. Spektralanal.* 1891, S. 7 et seq. This work gives a most excellent account of the best physical methods employed for the determination of colouring substances in solution. A useful review of methods up to date (1882) is given by Lambling, "Des procédés de dosage de l'hémoglobine," Nancy, 1882. Cf. later, E. von Fleischl, *Med. Jahrb.* 1885, S. 425. Malassez, *Arch. d. Physiol.* 1886, p. 257.

[5] For details see Gamgee, *Physiol. Chem.* Vol. I. p. 184.

ing power of a given thickness of the substance is determined once for all for a given region of the spectrum under given conditions, it becomes possible to determine the amount of that substance in any solution of unknown concentration by examining the solution under the same conditions in the same part of the spectrum and ascertaining how much light it has absorbed. Let I be the intensity of the incident light, and I' its reduced intensity after passing through m layers of a coloured solution, each of which reduces the initial intensity by $\frac{1}{n}$, then it follows that $$I' = \frac{I}{n^m}.$$
This is true whatever be the intensity of the incident ray; hence this intensity may be taken $= 1$, and we have $I' = \frac{1}{n^m}$.

Again, let E denote the reciprocal of the number which represents in centimeters that thickness of layer of the absorbing solution which reduces the intensity of the incident ray to $\frac{1}{10}$ of its initial intensity during its passage through this layer.[1] Then if the solution be examined in a layer which is always 1 cm. thick, this layer may be regarded as made up of E layers, each of thickness $\frac{1}{E}$ cm. Hence if in the formula previously given we put $n = 10$ and $m = E$, we find that the residual intensity I' of light after passing through a layer 1 cm. thick is $$I' = \frac{1}{10^E} = 10^{-E},$$
whence $$E = -\log.\ I'.$$

It can also be proved that E, the coefficient of extinction, is directly proportional to the amount of colouring matter present in the solution, or in other words, to its 'concentration;'[2] whence if the concentration be represented by C,

$$\frac{C}{E} = \text{some constant}^{3}\ A, \text{ or } C = AE.$$

This constant A having been determined once for all for a given substance in a solution of known concentration and for a *given region of the spectrum,* the concentration of any solution of the same substance of unknown strength is obtained by simply multiplying A by the coefficient of extinction E.[4]

Spectrophotometers are instruments by which the value of I' (see above) and hence of E may be determined. Those of Vierordt[5] and

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[1] E is called 'coefficient of extinction,' a term introduced by Bunsen and Roscoe, Pogg. *Annal.* Bd. CI. (1857), S. 235.

[2] The 'concentration' is the number of grams of colouring substance dissolved in 1 c.c. of fluid (Vierordt).

[3] Called the 'absorption ratio' by Vierordt.

[4] The introduction of the spectrophotometric method in a reliable form is due to Vierordt, based upon the photochemical researches of Bunsen and Roscoe. See Vierordt, (i) "Anwend. d. Spectralapparats zur Photometrie d. Absorptions-spectren u. z. quant. chem. Anal.," Tübingen, 1873, and (ii) "Die quant. Spectralanal. in ihrer Anwend. auf. Physiol. u. s. w.," Tübingen, 1876.

[5] *loc. cit.* (i), S. 52.

Hüfner[1] have been most generally used for physiological purposes, but there are many other forms.[2] The value of *A* has been determined by several observers for hæmoglobin,[3] oxy-hæmoglobin,[4] carbon-monoxide hæmoglobin[5] and methæmoglobin,[6] for certain fixed parts of the spectrum; as also its value for bile and urinary pigments.[7] If the value of *A* has been determined for two substances in *two* different parts of the spectrum, the amount of each substance in a mixture of the two may be determined spectrophotometrically.[8] This is a possibility of considerable importance when working with blood in which varying amounts of hæmoglobin and oxy-hæmoglobin may occur simultaneously.

6. **Methæmoglobin.** When blood or solutions of hæmoglobin which have been exposed to the air for some time are examined with the spectroscope they are frequently found to exhibit, in addition to the more or less persistent absorption bands of oxy-hæmoglobin, a marked band of absorption between *C* and *D*, closely resembling but differing slightly in position from the band which hæmatin shows in acid solution (see below). This band may also frequently be observed in many pathological fluids, such as those from ovarial cysts, etc., which are coloured by blood, and in urine when similarly coloured.[9] The substance to which the band is due is known as methæmoglobin.[10] It may be readily prepared in the laboratory by the action of many reagents such as acids or alkalis, or more conveniently of certain salts, on solutions of oxy-hæmoglobin. Of these salts those which may perhaps on the whole be most advantageously employed to obtain the spectrum of methæmoglobin are nitrites,[11] potassium ferricyanide, or potassium permanganate.[12] With the two latter salts the spectrum of methæmoglobin may be obtained as follows. To 10 c.c. of a moderately strong solution of oxy-hæmoglobin add a few drops of a dilute (·5—1·0 p.c.) solution of either of the salts and warm very gently. If on examination with a spectroscope the two bands of oxy-hæmoglobin are still strongly visible,

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[1] *Jn. f. prakt. Chem.* N.F. Bd. XVI. (1877), S. 290. Cf. Otto, Pflüger's *Arch.* Bd. XXXVI. (1885), S. 12. Glazebrook has constructed a modification of Hüfner's instrument. See Lea, *Jl. of Physiol.* Vol. V. (1883), p. 239.

[2] For all details of instruments and spectrophotometry in general see G. u. H. Krüss, *Kolorim. u. quant. Spektralanal.* 1891. Very complete details are given in Neubauer u. Vogel, *Analyse d. Harns*, 1891, S. 411.

[3] Hüfner, *Zt. f. physiol. Chem.* Bd. III. (1879), S. 7.

[4] Hüfner, *Ibid.* Bde. I. (1878), S. 317, III. (1879), S. 4. Von Noorden, *Ibid.* Bd. IV. S. 9. Otto, *Ibid.* Bd. VII. S. 62. Pflüger's *Arch.* Bd. XXXI. (1883), S. 244. XXXVI. (1885), S. 12. Sczelkow, *Ibid.* Bd. XLI. (1887), S. 373.

[5] Marshall, *Zt. f. physiol. Chem.* Bd. VII. (1882), S. 81.

[6] Otto, Pflüger's *Arch.* Bd. XXXI. (1883), S. 263.

[7] See Vierordt, *loc. cit.* or G. u. H. Krüss, *loc. cit.*

[8] Vierordt, *loc. cit.*

[9] Hoppe-Seyler, *Zt. f. physiol. Chem.* Bd. V. (1881), S. 6.

[10] The name was first used by Hoppe-Seyler in 1865, *Centralb. f. d. med. Wiss.* S. 65. But see also previously *Ibid.* 1864, S. 834, and Virchow's *Arch.* Bd. XXIX. (1864), Sn. 233, *u.* 597.

[11] Gamgee, *Phil. Trans.* 1868, p. 589.

[12] Jäderholm, *Zt. f. Biol.* Bd. XIII. (1877), S. 193.

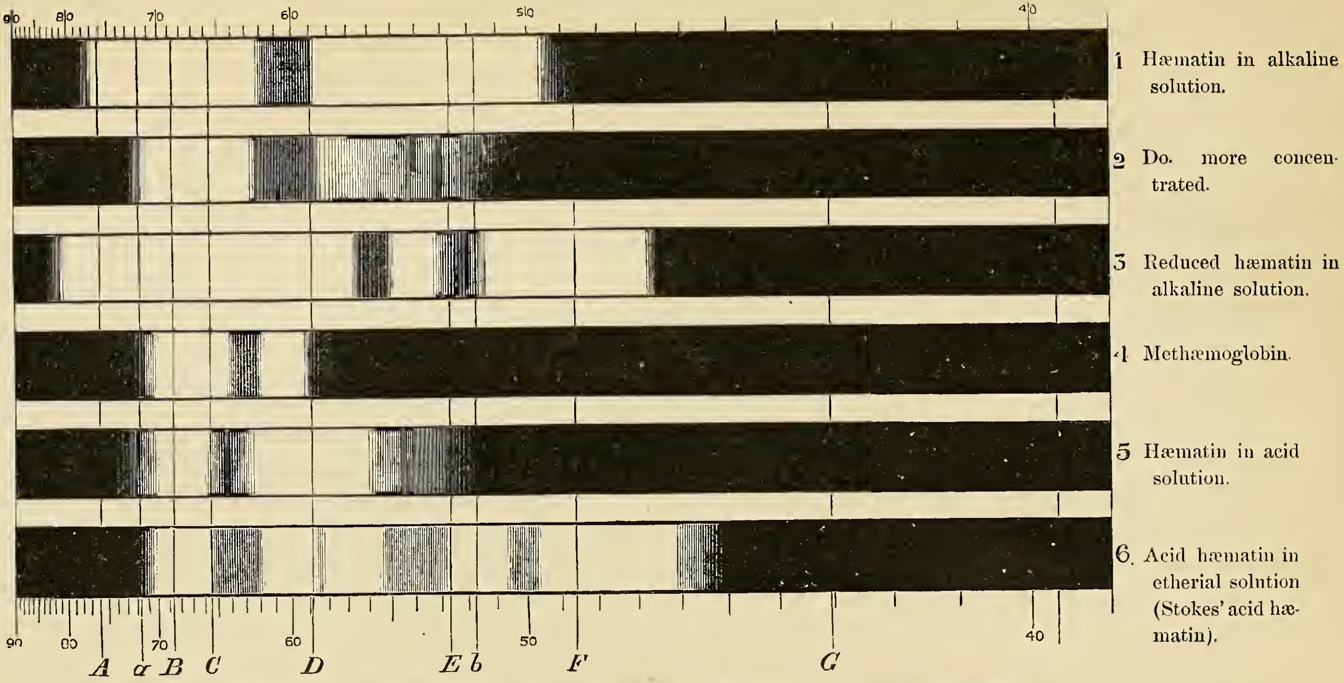
90
80
70
60
50
40
1 Hæmatin in alkaline solution.
2 Do. more concentrated.
3 Reduced hæmatin in alkaline solution.
4 Methæmoglobin.
5 Hæmatin in acid solution.
6. Acid hæmatin in etherial solution (Stokes' acid hæmatin).
A
α
B
C
D
E
b
F
G

Fig. 37. (After Gamgee.) Spectra of some derivatives of Hæmoglobin. Compare with Fig. 36.

let the mixture stand for a short time, and if the band characteristic of methæmoglobin has not made its appearance, add one or two drops more of the solution of the salt and proceed as before. As soon as the bands of oxy-hæmoglobin have markedly disappeared, acidulate very faintly and examine again. The band which should now be visible as characteristic of methæmoglobin lies in the red part of the spectrum, between *C* and *D*, nearer to the former line. As already remarked, its position is closely similar to that of hæmatin in acid solution; but comparison will show that it lies nearer *D* than does the hæmatin band, the centre of the latter being situated at W. L. 640, while that of the former is at W. L. 630[1] (See Fig. 37, Nos. 4 and 5).

In addition to the reagents recommended above, an extensive series of other substances are also found to effect the conversion of oxy-hæmoglobin into methæmoglobin, such as potasssium chlorate, amyl-nitrate, iodine dissolved in potassium iodide, bromine, osmic acid, hydrochinon, pyrocatechin, &c.[2] It may also be obtained as the result of prolonged evacuation with a mercurial pump, of putrefactive changes, or of the action of palladium saturated with hydrogen and immersed in the solution of oxy-hæmoglobin.[3]

The absorption band which has so far been described is the one which is to be regarded as characteristic of methæmoglobin, being accompanied by a very marked absorption of the violet end of the spectrum extending up to the *D* line. In addition to this band it is stated that, working with a good spectroscope of low dispersive power, three other bands may be additionally seen,[4] two corresponding closely with those of oxy-hæmoglobin but not identical, their centres corresponding to W. L. 580 and 539, and the third in the blue at W. L. 500 (?).[5]

In an alkaline solution the position of two of these bands differs slightly from that just given, being stated by Jäderholm to be at W. L. 602 and 578, while the third is unaltered at 539.

In the preparation of large quantities of crystallised oxy-hæmoglobin from pig's blood, it was observed that during the recrystallising essential to its purification a copious crop of reddish-brown crystalline needles was obtained. These were found on examination to be crystals of methæmoglobin.[6] They are most easily obtained if the oxy-hæmoglobin is first converted into methæmoglobin by the action of potassium ferricyanide (one or two minute crystals of the salt to half a litre of warm concentrated solution of oxy-hæmoglobin); the mixture is then shaken until it has a dark-brown colour and is cooled to 0° after the addition of one quarter of its bulk of alcohol also cooled to 0°. They have also been obtained from the blood of the dog,[1] horse,[2] and other animals,[3] and resemble in crystalline form the crystals of oxy-hæmoglobin from the same sources. These crystals are doubly refracting, readily soluble in water, though less so than oxy-hæmoglobin, and the solution, unlike that of the latter substance, yields a precipitate with basic lead acetate in presence of ammonia; they are identical in percentage composition with those of oxy-hæmoglobin. The behaviour of methæmoglobin towards reducing agents is interesting and also important as affording a means of discrimination between this substance and hæmatin. If some ammonium sulphide be added to an alkaline solution of methæmoglobin, the mixture may be observed to yield the spectrum of (reduced) hæmoglobin; and on now shaking up with oxygen (air) it shows the spectrum of oxy-hæmoglobin. When a solution of hæmatin is similarly treated it yields the spectrum of hæmochromogen (reduced hæmatin) in alkaline solution (see below). While the close relationship of methæmoglobin to oxy-hæmoglobin is thus clearly shown, very great differences of opinion have existed as to the exact nature of that relationship. Three views have been put forward. 1. That methæmoglobin is more highly oxidised than oxy-hæmoglobin. 2. That it is less highly oxidised. 3. That it is united with exactly the same amount of oxygen as is oxy-hæmoglobin, only in a more stable combination. The first view seems to have been based on the ready production of methæmoglobin by oxidising agents, and on the statement that when methæmoglobin is reduced it yields *first* oxy-hæmoglobin and then hæmoglobin. The second view rested on the possibility of obtaining methæmoglobin by the prolonged action of a vacuum or the shorter action of palladium saturated with hydrogen, and on the statement that by reducing agents it passes *at once* to hæmoglobin without the intermediate appearance of oxy-hæmoglobin. The third view, which now appears to be generally accepted, is derived from observations of the amount of oxygen which can be pumped out from a mixture of methæmoglobin and oxy-hæmoglobin of known composition,[4] and from the amount of oxygen which is displaced from a given weight of methæmoglobin when it is treated with nitric oxide.[1] We may probably say, therefore, that under certain conditions, without our being able to state exactly what has taken place, the oxygen loosely united to hæmoglobin as oxy-hæmoglobin becomes more stably combined, and is now not removable by either a vacuum, or carbon-monoxide, or a current of hydrogen, and further that the resulting substance (methæmoglobin) has the same composition and crystalline forms as oxy-hæmoglobin, and may be reconverted into the latter body by suitable means, such as reduction by ammonium sulphide and subsequent oxidation.

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[1] This method of localising the bands means that their centres occupy positions in the spectrum where the wave-length of light is respectively 640 and 625 millionths of a millimeter. It should always be adopted for all absorption bands, since it is independent of the varying dispersion and arbitrary scales of different spectroscopes. For details see Gamgee, *Physiol. Chem.* Vol. I. p. 94.

[2] For list of substances see Hayem, *Compt. Rend.* T. CII. (1886), p. 698.

[3] Hoppe-Seyler, *Zt. f. physiol. Chem.* Bd. II. (1878), S. 149.

[4] Jäderholm, *Zt. f. Biol.* Bd. XX. (1884), S. 419. Also *Nord. Med. Arkiv.* Abst. in Maly's *Jahresb.* 1884, S. 113. But see also Araki, *Zt. f. physiol. Chem.* Bd. XIV. (1890), S. 405.

[5] For figure see Halliburton, *Chem. Physiol. and Pathol.* Fig. 59, Spect. 6, p. 277.

[6] Hüfner u. Otto, *Zt. f. physiol. Chem.* Bd. VII. (1883), S. 65.

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[1] Hüfner, *Ibid.* Bd. VIII. (1884), S. 366. Jäderholm, *Zt. f. Biol.* Bd. XX. (1884), S. 419.

[2] Hammarsten, quoted by Jäderholm, *loc. cit.* S. 422.

[3] Halliburton, *Quart. Jl. Mic. Sci.* Vol. XXVIII. (1888), p. 201. Gives rapid method for microscopic purposes. See his *Chem. Physiol. and Pathol.* p. 280.

[4] The literature of the dispute is fully quoted and abstracted down to 1883 by Otto, Pflüger's *Arch.* Bd. XXXI. Sn. 245—255. The remaining literature to date (1892) has been given *passim* in the above account of this substance.

7. **Hæmocyanin.**[2] As previously stated (p. 217) the blood-plasma of many invertebrates contains hæmoglobin in solution; in a few cases this is united to special corpuscles in the blood. But in the case of other invertebrates this respiratory pigment is replaced by another to which, since it turns blue on exposure to air (oxygen), the name hæmocyanin has been given. Hence the arterial blood of those animals in which it occurs is blue, while the venous is colourless.

Hæmocyanin is a proteid of the globulin class; it is therefore partially precipitated by a current of carbon-dioxide, by saturation of its solutions with sodium chloride, and completely by saturation with magnesium sulphate.[3] Unlike hæmoglobin it has not yet been crystallised and contains copper, presumably as a constituent of its molecule, in place of the iron characteristic of hæmoglobin. It exhibits no absorption bands when examined spectroscopically.

Another animal pigment is known, into whose composition copper (5 — 8 p. c.) enters; this is the substance called turacin.[4] It gives the characteristic colour to the plumage of certain African birds known as Touracos or Plantain-eaters, whence the name turacin. It differs entirely from hæmocyanin in its general properties, and is only mentioned here because it contains copper, as does the former pigment. It is slightly soluble in water, readily soluble in dilute alkalis, the solutions in either of these solvents showing two absorption bands between *D* and *E* very similar to, though not identical with, the bands of oxy-hæmoglobin and a third faint broad band at *F*. It is not however a respiratory pigment.

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[1] Hüfner u. Külz, *Zt. f. physiol. Chem.* Bd. VII. (1883), S. 366.

[2] For literature see Halliburton, *Chem. Physiol. and Pathol.* 1891, p. 321. Details of previous work to date (1880) are given by Krukenberg, *Vergleich.-physiol. Studien*, III. Abth. (1881), S. 66.

[3] Halliburton, *Jl. of Physiol.* Vol. VI. (1884), p. 319.

[4] Church, *Phil. Trans.* Vol. CLIX. (1870), p. 627. Cf. *Ber. d. d. chem. Gesell.* Bd. II. (1869), S. 314; III. (1870), S. 459. See later Krukenberg, *Vergl.-physiol. Stud.* V. Abth. (1881), S. 72; 2 Reihe, I. Abth. (1881), S. 151. The same work (2 Reihe, Abth. II. u. III. 1882, Sn. 1 u. 128) contains elaborate observations on other pigments from feathers.

8. **Hæmochromogen.** $C_{34}H_{36}N_4FeO_5$ (?).

When (reduced) hæmoglobin is treated with acids, or, better still, with alkalis in the entire absence of oxygen, it is decomposed into a proteid and a coloured substance to which the name hæmochromogen was first given by Hoppe-Seyler.[1] When alkalis are used in its preparation, the solution obtained is of a brilliant purplish-red colour, and is characterised by two marked absorption bands, the stronger lying halfway between *D* and *E*, the other and fainter between *E* and *b*. These are identical with the bands of Stokes' reduced hæmatin in alkaline solution (see Fig. 37, No. 3). When exposed to the air (oxygen) the solution rapidly loses its brilliant colour, becomes dichroic, viz.: red in thick, and greenish in thin layers (cf. *sub* hæmatin) and now yields an absorption spectrum, which exhibits one not very strongly marked band in the yellow, to the red side of *D* and touching the latter line. This is the spectrum of hæmatin in an alkaline solution (see Fig. 37, Nos. 1 and 2). When the decomposition of the hæmoglobin is brought about by acids instead of alkalis, the coloured product is similarly hæmochromogen, but in this case, unless special precautions are taken, some of the hæmochromogen is itself further decomposed and yields hæmatoporphyrin or iron-free hæmatin (see below). The mixture thus obtained probably accounts for the four-banded spectrum as first described by Hoppe-Seyler.[2] When a solution of hæmatin in alkali is reduced with Stokes' fluid (see *sub* oxy-hæmoglobin) or ammonium sulphide the solution obtained shows two absorption bands identical with those already described as characteristic of hæmochromogen. From these facts it would at first sight appear that reduced hæmatin in alkaline solution and hæmochromogen in a similar solution are identical substances, and this is indeed the view which has been most generally adopted. From a spectroscopic point of view they do appear to be the same, but Hoppe-Seyler maintains that they are not.[3] According to him hæmochromogen is a simple product of the decomposition of hæmoglobin, while hæmatin is an oxidised product which differs from true oxy-hæmochromogen by being united to a smaller amount of oxygen than is the former. He has further succeeded in obtaining not only hæmochromogen in a crystalline form,[4] but also a compound of hæmochromogen with carbon-monoxide exhibiting the absorption bands of carbon-monoxide hæmoglobin and containing the same amount of carbon-monoxide united to each atom of iron as does that body, whereas hæmatin in alkaline solution will not unite with carbon-monoxide. He therefore considers that hæmoglobin is a compound of a proteid with this hæmochromogen, to which it owes its colour, and that it is with the hæmochromogen group rather than with hæmoglobin as a whole that the gases are united in the formation of such compounds as oxy-hæmoglobin and carbon-monoxide hæmoglobin. Further investigation, more particularly of the crystalline hæmochromogen, is needed for the final establishment of these views.

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[1] *Med.-chem. Unters.* Hft. IV. (1871), S. 540. Quoted in detail by Gamgee. *Physiol. Chem.* Vol. I. p. 118. See also later Hoppe-Seyler, *Zt. f. physiol. Chem.* Bd. I. (1877), S. 138.

[2] *Loc. cit.* Cf. Jäderholm (Swedish), Abst. in Maly's *Jahresb.* 1874, S. 104, 1876, S. 86. But see Hoppe-Seyler, *Physiol. Chem.* (1881), S. 394.

[3] *Zt. f. physiol. Chem.* Bd. XIII. (1889), S. 477.

[4] By the action of strong caustic soda at 100° in the entire absence of oxygen.

9. **Hæmatin.** $C_{34}H_{35}N_4FeO_5$.[1]

When oxy-hæmoglobin is decomposed by either acids or alkalis it yields a proteid and a coloured substance known as hæmatin. This decomposition may take place in old blood-clots or extravasations and is readily produced by the action of either gastric or pancreatic juice on oxy-hæmoglobin, so that hæmatin is frequently found in the contents of the alimentary canal and in the fæces, more especially with a flesh diet. It has also been found in urine as the result of poisoning with sulphuric acid or arseniuretted hydrogen.

Preparation. The following method slightly modified after Kühne[2] may be advantageously employed, and yields not only solutions which show strikingly the spectroscopic appearances of hæmatin in acid and alkaline solution, but also finally a fairly pure and typical specimen of hæmatin itself. Defibrinated blood is made into a thin paste by mixture with potassium carbonate, and is then evaporated to dryness on a water-bath. The dry residue is powdered, placed in a flask, and extracted with about four times its bulk of strong alcohol by boiling on a water-bath. The deeply coloured extract thus obtained is poured off and the residue again extracted as before with alcohol, the process being repeated as long as any colouring matter is extracted. The extracts are mixed and filtered and form a strong solution (*a*) of hæmatin in alkaline alcohol. A portion of this extract may be kept for spectroscopic examination. The remainder is strongly acidulated by the careful addition of sulphuric acid, any precipitate which is formed is removed by filtration, and the filtrate (*b*) provides a typical solution of hæmatin in acid alcohol. A portion of this may as before be kept for spectroscopic examination. The remainder is made alkaline by the addition of an excess of ammonia and filtered; the filtrate (*c*) is, as in the case of (*a*), a solution of hæmatin in alkaline alcohol, but now the extraneous salts present are chiefly those of ammonium. The filtrate (*c*) is finally evaporated to dryness on a water-bath, extracted with several portions of boiling water, and the undissolved residue consists of fairly pure hæmatin. This should finally be washed with alcohol and ether and then dried for a prolonged period at 130–150°.

To obtain pure hæmatin it is probably better to prepare it from hæmin whose purity as a mother substance can be ensured at the outset by the fact that, unlike hæmatin, it is readily obtained in crystals. (See below.) The hæmin crystals should be boiled with strong acetic acid, then washed with water, alcohol, and ether, and dissolved in dilute caustic potash. The solution is then filtered, precipitated with hydrochloric acid, and washed with boiling water until the washings are shown, as tested by nitrate of silver, to be free from hydrochloric acid. The residue is finally dried by prolonged heating to 130—150°.[1]

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[1] Hoppe-Seyler, *Med.-chem. Untersuch.* 1871, Hft. 4, S. 523.

[2] *Physiol. Chem.* 1868, S. 202.

For ordinary purposes hæmatin is characterised chiefly by the spectroscopic appearances of its solutions. When dissolved in an alkali (ammonia, as in solution (*c*) above) it shows one absorption band in the yellow adjoining *D* to the red side of this line, while at the same time there is great absorption at the blue end of the spectrum (Fig. 37, Nos. 1 and 2). On treatment with a reducing agent, Stokes' fluid or ammonium sulphide, this band is replaced by two others in the green, of which the one nearest *D* is remarkably dense, the other less sharply defined. Very little absorption of the red end is observed while that of the blue is as before very marked (Fig. 37, No. 3). This is the spectrum of Stokes' reduced hæmatin and is identical with that of Hoppe-Seyler's hæmochromogen. The two substances have usually been regarded as identical, but this is disputed by Hoppe-Seyler (see above). Alkaline solutions of hæmatin are strongly dichroic, being ruby-red in thick layers and greenish in thin layers viewed by reflected light.

The acid alcoholic solution of hæmatin (solution (*b*) above) is characterised by one absorption band between *C* and *D*, adjoining *C*, whose centre is situated at W. L. 640. This band is somewhat similar to that of methæmoglobin, but it is less dense, and careful observation shows that the centres of the respective bands do not coincide (Fig. 37, Nos. 5 and 4). Acid solutions of hæmatin are monochromatic and of a dull reddish-brown colour. If blood or a strong solution of oxy-hæmoglobin be made strongly acid by the addition of acetic acid the hæmoglobin is decomposed, hæmatin is set free, and if the solution be shaken up with ether and allowed to stand, the ether rises to the surface and is more or less coloured owing to the presence of hæmatin held in solution in the acid ether. This acid ethereal solution shows, in addition to the one band already described as characteristic of hæmatin in an acid solution, three other bands whose positions and relative intensities are sufficiently shown in Fig. 37, No. 6.

Hæmatin as prepared by the methods described above is usually obtained as a scaly but not crystalline mass of bluish-black colour and metallic lustre, strongly resembling iodine. When finely powdered it appears dark or light-brown according to the fineness of the powder. It is a remarkably stable substance; may be heated to 180° without decomposition, but by stronger heating is finally decomposed, liberates an odour of hydrocyanic acid, and leaves a residue (12·5 p. c.) of pure oxide of iron. It is quite insoluble in either water, alcohol, ether, chloroform, or benzol. It is somewhat soluble in strong acetic acid, especially if warm, also in alcohol (not water) to which some acid has been added, and readily soluble in alkaline solutions or in alcohol containing alkalis. It is not affected either by strong caustic alkalis even when heated, or by hydrochloric or nitric acids. It may be dissolved in strong sulphuric acid, but is now found to have undergone a change during solution which results in the removal of iron and the production of hæmatoporphyrin or iron-free hæmatin[1] (see below).

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[1] Hoppe-Seyler, *Physiol.-pathol.-chem. Anal.* 5 Aufl. 1883, S. 239. See also Cazeneuve, *Thèse*, Paris, 1876, Abstr. in Maly's *Jahresb.* 1876, S. 76. *Bull. Soc. Chim.* T. XXVII. (1877), p. 485. MacMunn, *Jl. of Physiol.* Vol. VI. 1884, p. 22.

If the decomposition of hæmatin by sulphuric acid be brought about in the absence of oxygen an iron-free insoluble substance is obtained known as hæmatolin, to which the formula $C_{68}H_{78}N_8O_7$ is assigned.[2]

If potassium cyanide be added to an alkaline solution of hæmatin, this now shows one broad absorption band extending from D to E (Hoppe-Seyler). By the action of reducing agents, this band is replaced by two other bands.[3] The substance to which these appearances are due is known as cyan-hæmatin, but all further information is still wanting.

Some more recent observers (Nencki and Sieber) have assigned to hæmatin the formula $C_{32}H_{32}N_4FeO_4$, the validity of which as against the views of Hoppe-Seyler is not as yet generally accepted. It will be referred to again under hæmin.

10. **Histohæmatins.** This is the name assigned to a class of pigments which are stated to be of wide-spread occurrence in the tissues of both vertebrates and invertebrates, and to be related to though quite distinct from hæmoglobin and hæmatin. They are regarded as respiratory pigments, playing towards the muscles or other tissues in which they more particularly occur the same part that hæmoglobin does to the tissues generally. Our knowledge of these pigments is however as yet limited to the spectroscopic appearances which they present either *in situ* in the mother-tissue or in solutions obtained by the action of ether, while their respiratory function is assumed from the changes which they exhibit under the influence of reducing agents and subsequent exposure to oxygen. Of these histohæmatins the one most fully described is known as myohæmatin from its characteristic presence in muscles.

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[1] The hæmatoin of Preyer. See "Die Blutkrystalle," 1871, S. 178.

[2] Hoppe-Seyler, *Med.-chem. Unters.* 1871, Hf. 4, S. 533. Cf. Nencki u. Sieber, *Ber. d. d. chem. Gesell.* Bd. XVII. (1884), S. 2272.

[3] See Gamgee, *Physiol. Chem.* Vol. I. p. 115.

Myohæmatin.[1] To observe the spectrum of this substance a slice of tissue, such as that of the heart, is squeezed in a compressorium until sufficiently thin to transmit light. It is then examined with a microspectroscope under strong illumination. Or, on the other hand, the tissue may be treated with excess of ether under whose influence an aqueous juice is extruded in which the myohæmatin is in solution. Speaking generally, for the appearances vary slightly according to the source of the pigment, myohæmatin yields a four-banded absorption spectrum. The first band lies close to *D*, but towards the red end of the spectrum. The next two bands are situated close together about midway between *D* and *E*. The remaining band lies in the region between *E* and *b*. Solutions of myohæmatin are when weak of a reddish-yellow colour, but if strong they are pure red. By the action of warm alcohol containing a little sulphuric acid a spectrum is obtained closely similar to that of hæmatin in acid solution, and by the use of concentrated sulphuric acid a substance is produced which in both acid and alkaline solutions shows bands similar to those of hæmatoporphyrin in the same solvents. Under certain conditions myohæmatin becomes 'modified' and now yields two bands similar to those of hæmochromogen, but situated nearer the violet end of the spectrum.

The conclusions drawn from the above spectroscopic facts have been the subject of some controversy and adverse criticism, the appearances being regarded as due not to a specific pigment, but rather to hæmochromogen or mixtures of other products of the decomposition of hæmoglobin.[2]

11. **Hæmin.** $C_{34}H_{35}N_4FeO_5 . HCl$. (Hæmatin-hydrochloride, or Teichmann's crystals.)

These crystals may be readily obtained for microscopic examination by heating a drop of fresh blood on a glass-slide under a cover-slip with a little glacial acetic acid.[3] In the case of blood which has been dried, as in an old blood-clot or stain, the

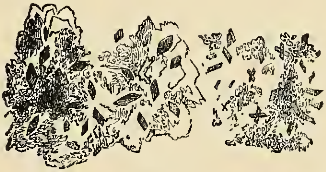

FIG. 38. HÆMIN CRYSTALS FROM A DROP OF BLOOD. (Kühne.)

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[1] MacMunn, *Phil. Trans.* Pt. I. 1886, p. 267, *Jl. of Physiol.* Vol. VIII. (1887), p. 51.

[2] Levy, *Zt. f. physiol. Chem.* Bd. XIII. (1889), S. 309. Hoppe-Seyler, *Ibid.* Bd. XIV. (1890), S. 106. For reply see MacMunn, *Ibid.* XIII. S. 497, XIV. 328.

[3] Teichmann, *Zt. f. rat. Med.* Bd. III. (1853), S. 375, Bd. VIII. S. 141.

residue should be powdered as finely as possible with a minute quantity (trace) of sodium chloride. A little of the powder is then placed on a slide and covered with a slip under which some glacial acetic acid is now run in. It is then warmed carefully to a temperature just short of that which would cause the acid to boil. If the operation has been successful, on cooling crystals of hæmin will be seen under a microscope, mixed in either case as in Fig. 38 with a granular débris. If they are absent, warm again, adding more acid if necessary. The crystals are dark-brown, frequently almost black, elongated rhombic plates and prisms belonging to the triclinic system.[1] In a purified specimen they are arranged singly or in groups as shown in Fig. 39, and apart from their form are characterised by being strongly doubly-refracting: when examined under the microscope between crossed Nicol prisms those crystals whose axes are suitably inclined to the incident light stand out bright yellow or orange on the dark field.[2] They are quite insoluble in either water, alcohol, ether, chloroform, or dilute acids: they may however be dissolved to some extent in glacial acetic or hydrochloric acids, especially if warmed, and are readily soluble in alkaline carbonates or dilute caustic alkalis, being at the same time decomposed by the latter solvent into hæmatin and a chloride of the alkali. This fact provides the best means for obtaining pure hæmatin (see above).

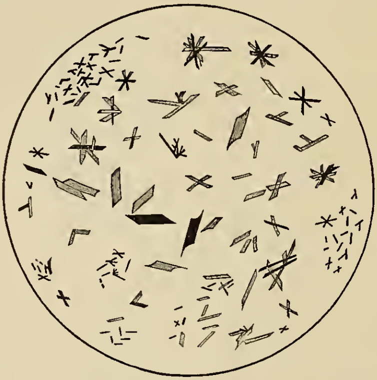

Fig. 39. Hæmin crystals. (After Preyer.)

Although it is quite easy to obtain typical crystals under the microscope from minute amounts of hæmoglobin or hæmatin, their preparation on a large scale is somewhat tedious; several methods have been employed,[1] of which the most recent, said to yield 5 gr. of crystals from each 1 litre of blood, is as follows.[2] To each volume of defibrinated and strained blood add four volumes of glacial acetic acid previously warmed to 80°. As soon as the temperature of the mixture has fallen to 55 — 60°, it must be again warmed to 80°. On cooling and standing for 10 — 12 hours crystals separate out; the supernatant liquid is then removed by a syphon, the crystals are washed with water repeatedly by decantation in a tall glass cylinder and are finally collected on a filter and washed with water, alcohol, and ether.

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[1] Lahorio. Quoted by Schalfejew, *Jn. d. russ. phys.-chem. Gesell.* 1885, S. 30. See Abstr. in *Ber. d. d. chem. Gesell.* Bd. XVIII. Ref., S. 232. Cf. Högyes, *Centralb. f. d. med. Wiss.* 1880, No. 16.

[2] A. Ewald, *Zt. f. Biol.* Bd. XXII. (1886), S. 474.

The successful preparation of hæmin crystals from minute quantities of hæmoglobin or methæmoglobin is of the greatest importance for medico-legal purposes, since they suffice, even in the absence of all other confirmatory evidence, to establish the nature of the material used in their preparation. In the detection of blood-stains it is usual first to examine with a spectroscope an aqueous solution of the colouring matter if it can be obtained, for the characteristic absorption bands of oxy-hæmoglobin or methæmoglobin. In old stains the hæmoglobin is frequently decomposed, in which case it is insoluble in water, and alkaline extracts must be made and examined for the spectra characteristic of hæmatin. The residues from the spectroscopic examination are lastly used to prepare hæmin crystals, in final confirmation of the evidence previously obtained.[3]

Allusion has already been made (see p. 234) to some work on hæmin and hæmatin which assigns to these substances a composition and relationship very different from those usually accepted, and further puts the relationship of the colouring matter of blood to the bile-pigments in a new light.[4] With the preliminary caution that these views are not as yet generally accepted and require confirmation, they may be briefly dealt with here. Using amyl-alcohol in the preparation of hæmin crystals it is stated that the crystals have the following composition $(C_{32}H_{30}N_4FeO_3 . HCl)_4 C_5H_9 . OH$. The group $C_{32}H_{30}N_4FeO_3$ is regarded as the true hæmin, Teichmann's crystals consisting of $C_{32}H_{30}N_4FeO_3 . HCl$. When the crystals thus prepared are decomposed by caustic alkalis as in the ordinary method for preparing hæmatin from them, the hæmin is supposed to take up one molecule of water and yield hæmatin $C_{32}H_{32}N_4FeO_4$. By treating this hæmatin with strong sulphuric acid, it loses its iron and uniting with oxygen yields hæmatoporphyrin or iron-free hæmatin, $C_{32}H_{32}N_4O_5$, which is however further regarded as derived by dehydration from a true hæmatoporphyrin whose composition is $C_{16}H_{18}N_2O_3$. The latter is thus identical in composition with bilirubin, whose formula is undoubtedly $C_{16}H_{18}N_2O_3$. This is regarded as affording the desired chemical proof of the genetic relationship of the bile- and blood-pigments, the derivation of the former from hæmatin being represented as follows, $C_{32}H_{32}N_4FeO_4 + 2H_2O - Fe = 2\,(C_{16}H_{18}N_2O_3)$.

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[1] See Gamgee, *Physiol. Chem.* Vol. I. p. 116, or Hoppe-Seyler, *Physiol. pathol.-chem. Anal.* Aufl. 5, 1883, S. 241.

[2] Schalfejew, *Jn. d. russ. phys.-chem. Gesell.* 1885. See Abstr. in *Ber. d. d. chem. Gesell.* XVIII. Bd. (1885), Ref., S. 232.

[3] For details see Hoppe-Seyler, *loc. cit.* S. 529. Gamgee, *loc. cit.* p. 217. MacMunn, *The spectroscope in medecine*, 1883, pp. 130—148.

[4] Nencki u. Sieber, *Ber. d. d. chem. Gesell.* Bd. XVII. (1884), S. 2267, XVIII. S. 392, *Arch. f. exp. Path. u. Pharm.* Bd. XVIII. (1884), S. 401, Bd. XX. (1886), S. 325. Bd. XXIV. (1888), S. 430. Nencki u. Rotschy. *Monatshf. f. Chem.* Bd. X. (1889), S. 568. See also Hoppe-Seyler in adverse criticism, *Ber. d. d. chem. Gesell.* Bd. XVIII. (1885), S. 601, *Zt. f. physiol. Chem.* Bd. X. (1886), S. 331.

12. **Hæmatoporphyrin.**[1] $C_{68}H_{74}N_8O_{12}$ (?). (Iron-free hæmatin.)

If hæmatin is dissolved in concentrated sulphuric acid it yields a solution which, after filtration through asbestos, is of a brilliant purple-red colour. By the action of the acid, the iron is removed from the hæmatin and hæmatoporphyrin is formed.[2] If this solution is diluted with sulphuric acid it shows with a spectroscope two absorption bands of which one adjoins *D* to the red side of this line, while the other is very strongly marked and lies midway between *D* and *E*. By the addition of water to the solution in sulphuric acid the colouring matter is largely precipitated, especially if some alkali be carefully added to neutralise the acid. The precipitate thus obtained is readily soluble in dilute alkalis, and this solution is characterised by four absorption bands, one halfway between *C* and *D*, two between *D* and *E*, and one conspicuous band adjoining *b* and extending nearly to *F*.[3] Hæmatin also yields hæmatoporphyrin by the action of strong hydrochloric acid at 130° in sealed tubes.

Some interest attaches to this substance owing to its occasional occurrence in urine in forms which show slightly different absorption spectra but are probably closely related if not identical. Thus it occurs as urohæmatin or urohæmatoporphyrin,[4] or as ordinary hæmatoporphyrin.[5] It is also found in the integument of some invertebrates[6] and in the egg-shells of certain birds.[7] It is further interesting to notice that in hæmatoporphyrin we have a strongly coloured pigment derived from hæmatin with removal of the iron which the latter contains, a fact which facilitates our conception of a possible derivation of the iron-free bile-pigments from the iron-containing hæmoglobin or hæmatin. This relationship will be more fully discussed when the bile-pigments are described.

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[1] Hoppe-Seyler, *Med.-chem. Unters.* Hft. 4, 1871, S. 528.

[2] In the absence of oxygen a substance called by Hoppe-Seyler hæmatolin is obtained, $C_{68}H_{78}F_8O_7$.

[3] These spectra are figured in Halliburton, *Chem. Physiol. and Pathol.* Fig. 59, p. 277, Nos. 10 and 11.

[4] MacMunn, *Proc. Roy. Soc.* Vol. XXXI. 1880, p. 206, *Jl. of Physiol.* Vols. VI. (1884), p. 36, X. (1889), p. 71, *Clinical Chem. of Urine*, 1889, p. 109, *Proc. Physiol. Soc.* No. IV. 1890. See *Jl. of Physiol.* Vol. XI. (1890), p. xiii.

[5] E. Salkowski, *Zt. f. physiol. Chem.* Bd. XV. (1891), S. 286.

[6] MacMunn, *Jl. of Physiol.* Vols. VII. (1885), p. 240, VIII. p. 384.

[7] For literature see MacMunn, *Jl. of Physiol.* Vol. VII. p. 251.

13. **Hæmatoidin.**[1] $C_{16}H_{18}N_2O_3$.

This substance is found as reddish or orange rhombohedral crystals in old blood-clots as of cerebral hæmorrhages,[2] in corpora lutea, in the urine in cases of transfusion of blood[3] and in cases of hæmaturia.[4] There is no doubt that as occurring in the above cases it is directly derived from some metamorphosis of hæmoglobin. Apart from the similarity of crystalline form and colour it was further found that hæmatoidin crystals readily give the characteristic (Gmelin's) reaction for bilirubin by treatment with nitric acid, and thus its identity with bilirubin was at once asserted and supported by very convincing evidence.[5] The identity was however for some time disputed, notably by Städeler, and by others largely on the basis of inconclusive spectroscopic investigation of the two substances. There is however no doubt that hæmatoidin is really identical with bilirubin, so that now the name is of interest rather from a historical point of view and physiologically as indicating the undoubted genetic relationship of the pigments of bile to those of blood.

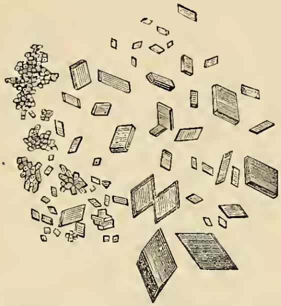

FIG. 40. HÆMATOIDIN CRYSTALS. (Frey after Funke.)

BILE-PIGMENTS AND THEIR DERIVATIVES.[6]

The bile is in all animals a characteristically highly-coloured secretion. The colour of the *fresh* bile is as a general rule golden-red in man and carnivora, and more or less bright green in herbivora. These colours are due to the presence of a pigment known as bilirubin in the first case and biliverdin in the second; but since the latter pigment may be readily formed by simple oxidation from the former, bile may frequently contain both these colouring-matters and hence possess a colour intermediate to the above though usually with a preponderance of either the golden-red or green shade. In addition to these two pigments others are occasionally present in bile, as evidenced by the fact that while neither bilirubin nor biliverdin exhibits any absorption bands when examined spectroscopically, fresh bile of herbivora[1] frequently does show bands, due to substances of which but little is known beyond these spectroscopic appearances (see below). It is possible that the bile-pigments of different animals may ultimately be found to differ slightly but distinctly in their composition, much in the same way that the bile-acids as already stated differ; but as yet no such distinct differences have been made out, and we may therefore treat of them as being identical from whatever source they have been obtained.

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[1] The literature of this substance is very fully quoted in Hermann's *Hdbch. d. Physiol.* Bd. v. Th. 1, S. 245.

[2] Virchow first carefully described it as obtained from this source, and named it hæmatoidin to indicate its undoubted derivation from the colouring matter of the blood. Virchow's *Arch.* Bd. I. (1847), S. 419.

[3] Hoppe-Seyler, Pflüger's *Arch.* Bd. x. (1875), S. 211.

[4] Ebstein, *Deutsch. Arch. f. Klin. Med.* 1878, S. 115.

[5] See among others E. Salkowski, Hoppe-Seyler's *Med.-chem. Unters.* Hf. 3, 1868, S. 436.

[6] See specially Maly in Hermann's *Hdbch. d. Physiol.* Bd. v. Th. 2, 1881, S. 154. Also, for history and literature, Heynsius u. Campbell, Pflüger's *Arch.* Bd. IV. (1871), S. 497.

1. **Bilirubin.** $C_{16}H_{18}N_2O_3$.[2]

It occurs chiefly and characteristically in the *fresh* bile of man and carnivora, to which it imparts the well-known golden-red colour. It frequently constitutes the larger part of some kinds of gall-stones, more especially of the ox and pig, not as free bilirubin but as a compound with chalk, and amounting to some 40 p.c. of the concretions. (Maly.)[3] It is also found in the urine in icterus, also constantly in the serum from horses' blood, though not from that of man or the ox,[4] and frequently as crystals under the name 'hæmatoidin' (see above) in old blood-clots (extravasations) and fluids from ovarial and other cysts. Bile-pigments are also stated to occur normally in the urine of dogs, more particularly in the summer.[5]

Bilirubin is insoluble in water and almost insoluble in either ether or alcohol, though distinctly more soluble in alcohol than in ether. It is on the other hand readily soluble in alkaline solutions, hence its solution in bile, also in glycerin carbon-disulphide, and benzol, and above all in chloroform. From its solution in the latter it may be separated out by extremely slow evaporation of the solvent in a crystalline form as rhombic plates or prisms. The general shape of these is shown above in Fig. 40; but as obtained from solution in either carbon-disulphide or chloroform the crystals usually exhibit somewhat blunt ends and slightly convex surfaces as first pointed out by Staedeler. As ordinarily prepared it is an amorphous powder of the colour of sulphide of antimony. It readily forms compounds with bases, *e.g.* sodium, barium, and calcium, the latter providing a convenient means for the separation of bilirubin from bile, urine, or other dilute solution.

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[1] Bile of carnivora does not usually show bands until it has been treated with an acid.

[2] This is the generally accepted formula, assigned to this substance by Maly. *Jn. f. prakt. Chem.* Bd. CIV. (1868), S. 28, confirming Staedeler. It is possible that the formula is really twice the above, viz. $C_{32}H_{33}N_4O_6$, as required to represent the formula of a well-defined tribromo-substitution product, $C_{32}H_{33}Br_3N_4O_6$. This doubling of the formula is also necessary to express the derivation of hydrobilirubin. ($C_{32}H_{40}N_4O_7$) from bilirubin. Maly, *Sitzb. d. k. Akad. d. Wiss. Wien.* III. Abth. Oct.-Hft. 1875. Liebig's *Annal.* Bd. CLXXXI. (1876), S. 106.

[3] See earlier Staedeler, *Vierteljahrschr. d. naturforsch. Gesell.* Zürich, Bd. VIII. 1863, and Liebig's *Annal.* Bd. CXXXII. (1864), S. 323.

[4] Hammarsten (Swedish). See Abstr. in Maly's *Jahresb.* 1878, S. 129.

[5] Salkowski u. Leube, *Die Lehre vom Harn*, 1882, S. 246.

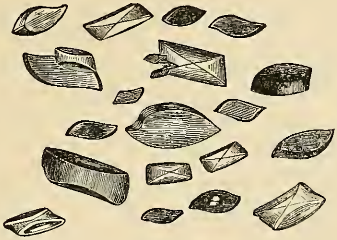

Fig. 41. Bilirubin Crystallised from Carbon-disulphide. (Krukenberg.)

Preparation. (i) When gall-stones are not available bile may be treated as follows.[1] The bile is slightly diluted with water, some lime-water is added (avoiding excess) and after thorough mixture, as by shaking, a current of carbon dioxide is passed to convert all the excess lime into carbonate. The precipitate thus formed contains the bilirubin as a calcium compound. This is then collected on a filter, washed with water, and after suspension in a little water, decomposed by the addition of a slight excess of acetic or hydrochloric acid. By this means the bilirubin is set free, and may now be extracted by shaking with an excess of chloroform. The chloroform solution is separated by decantation, and evaporated to a small bulk; the bilirubin may then finally be precipitated by an excess of alcohol. The amount thus obtained is not quantitatively accurate, since all the bilirubin is not precipitated by the lime at the outset and there is a further loss during the subsequent operations. (ii) Since, as already stated, the gall-stones of the ox or pig may consist of nearly half their weight of bilirubin combined with calcium, they provide the best and simplest source for the preparation of this substance.[1] The stones are finely powdered, extracted with ether to remove any cholesterin, then with water and treated with either strong acetic acid or dilute hydrochloric acid. By this means the bilirubin is set free from its calcium compound, and after being washed with water and alcohol is dissolved in chloroform, and finally separated by precipitation with alcohol as already described. To obtain it quite pure the dissolving in chloroform and precipitating by alcohol should be repeated several times. The final product is amorphous. Crystals are most readily obtained by slow evaporation of the first and hence slightly impure solution in chloroform.

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[1] Based on Huppert, *Arch. d. Heilk.* Bd. VIII. (1867), S. 345, 476. See Hoppe-Seyler, *Hdbch. d. physiol.-path. chem. Anal.* 1883, S. 250. Cf. Hilger, *Arch. d. Pharm.* (3), Bd. VI. (1875), S. 385.

When carnivorous bile is exposed to the air it turns more or less rapidly green; this is due to its oxidational conversion into biliverdin, the normal pigment of herbivorous bile. A similar change is at once produced by an oxidising agent such as nitric acid containing nitrous acid, but in this case the change of colour does not stop short with green, but passes successively through blue, violet, and red to a final yellow. These later colours are due to products of the progressive oxidation of the first formed biliverdin, but with the exception of the final substance (choletelin) are as yet but imperfectly characterised. The play of colours observed when either bilirubin or biliverdin is oxidised, constitutes the well-known Gmelin's reaction.[2] This is extremely delicate and may be applied in either of the two following ways. A few drops of the suspected solution are placed on a porcelain slab and a drop of yellow fuming nitric acid is brought into contact with it. A play of colours is observed at the junction of the fluids if bile-pigments are present. Or on the other hand some of the acid may be poured into the bottom of a test-tube and the suspected fluid carefully added so as not to mix with the acid but float on its surface. If bile-pigments are present coloured rings (layers) appear at the junction of the two liquids, being yellow nearest the acid and progressively red, violet, blue, and green passing upwards. It is stated that this test will detect as little as 1 part of bilirubin in 70,000 — 80,000 parts of solvent.

Other tests have been recommended, but they are perhaps unnecessary in view of the extreme delicacy of Gmelin's reaction when properly applied.[3] The certain detection of minute amounts of bile-pigments in urine is frequently of great clinical and physiological importance. If any very appreciable quantity of the pigments are present, Gmelin's reaction applied as above will usually suffice to detect them. If not they may be obtained in a more concentrated residue, which has been largely freed by Huppert's method from other colouring matters which interfere with the test. The fluid is precipitated by lime-water and carbon dioxide. The compound of lime and bilirubin is then collected on a filter, washed and tested *in situ* by the addition of fuming nitric acid; or it may be boiled in a test-tube with a little alcohol acidulated with sulphuric acid; the precipitate loses its colour and the supernatant alcohol turns to a brilliant green. The following is also a reliable test as applied to urine.[1] To 20 or 30 c.c. of urine add 5 to 10 c.c. of a solution of zinc acetate (1:5). This causes a voluminous precipitate of bile-pigments, especially if the acid reaction be somewhat reduced by the simultaneous addition of a little sodium carbonate. The precipitate is collected on a filter, washed with water, and dissolved in a little ammonia. If bile-pigments are present the solution is usually fluorescent, and on standing, if not at once, shows the absorption bands characteristic of bilicyanin. (See below.) For further details of other methods consult some special work.[2]

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[1] The coloured residue from human gall-stones left after the extraction of cholesterin (p. 131) may also be used for the preparation of bilirubin.

[2] Tiedemann u. Gmelin. Die Verdauung nach Versuchen, 1826, S. 80.

[3] See more particularly Capranica, *Gaz. chim. Ital.* Vol. XI. (1881), p. 430. Moleschott's *Untersuch. z. Naturlehre*, Bd. XIII. (1882), S. 190. Ehrlich. *Centralb. f. klin. Med.* 1884, No. 45, or *Centralb. f. d. med. Wiss.* 1884, S. 143. In the latter case a solution of diazobenzosulphonic acid is employed, and is stated to discriminate between bilirubin and other bile-pigments.

The accurate quantitative determination of bilirubin, as of other bile, and also of urinary-pigments is only possible by spectrophotometric methods. These have been already briefly described on p. 224. The requisite constants for the application of the method in the case of each pigment are given in the literature quoted below.[3]

Bilirubin, while it exhibits no distinct absorption bands, is characterised by a powerful absorption of the violet end of the spectrum.

2. **Biliverdin.** $C_{16}H_{18}N_2O_4$.

This is, as already stated, the first product of the oxidation of bilirubin. It gives the characteristic colour to the bile of herbivora, probably accounts for the colour of biliary vomit in carnivora (man), is possibly found in the urine in icterus, has been stated to occur in the edges of the placenta in pregnant animals[4] (bitches), while on the other hand it occurs in mere traces in gall-stones whether of man or other animals. It has also been described as occurring in egg-shells[5] and the integuments of certain invertebrates.[6]

Preparation. An impure product may be obtained as follows from herbivorous bile. After the removal of mucin (p. 76), barium chloride is added; this precipitates the pigment as a compound with barium (?). The precipitate is then collected on a filter, washed with water and alcohol, and decomposed with dilute hydrochloric acid; this liberates the biliverdin which is simultaneously precipitated as a flocculent mass, and is then washed with ether to remove all fat and dissolved in alcohol. The alcoholic solution is finally filtered and by spontaneous evaporation yields a dark-green glittering residue of impure biliverdin. To obtain the pigment pure it must be prepared from bilirubin. The conversion may be effected in several ways.[1] (i) Bilirubin is dissolved in a dilute alkali and exposed for some time to the air in thin layers, whereby it is slowly oxidised into biliverdin. When the conversion is complete, the pigment is precipitated by the addition of hydrochloric acid, thoroughly washed with water, dissolved in absolute alcohol, and precipitated by an excess of water or by spontaneous evaporation of the alcoholic solution. (ii) By enclosing bilirubin solutions in tubes with glacial acetic acid and heating to 100°. (iii) By the action of monochloracetic acid and gentle heating at intervals for one or two days. (iv) Also by the action of caustic potash on tribromobilirubin.[2]

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[1] Stokvis. See Abst. in Maly's *Jahresb.* 1882, S. 226.

[2] Neubauer u. Vogel, *Anal. d. Harns*, 1890, S. 321 *et seq.*

[3] Vierordt, *Die quant. Spectralanalyse u. s. w.* Tübingen, 1876, S. 76. *Zt. f. Biol.* Bd. IX. (1873), S. 160, Bd. X. (1874), S. 21, 399. Vossius, *Arch. f. exp. Pathol.* Bd. XI. (1879), S. 427.

[4] Etti. See Maly's *Jahresb.* 1871, S. 233, and 1872, S. 287.

[5] Liebermann, *Ber. d. d. chem. Gesell.* Bd. XI. (1878), S. 601. Krukenberg, *Verhandl. d. physik.-med. Gesell. zu Würzburg*, Bd. XVII. (1883), S. 109.

[6] Krukenberg, *Centralb. f. d. med. Wiss.* 1883, S. 785.

Apart from its colour biliverdin differs most characteristically from bilirubin in its solubilities. It is (like bilirubin) soluble in alkalis but insoluble in water and ether, whereas (unlike bilirubin) it is insoluble in either chloroform, carbon bisulphide or benzol, but readily soluble in alcohol and in glacial acetic acid. It has further never been obtained in a crystalline form, and like bilirubin it shows no absorption bands but a somewhat strong absorption of the violet end of the spectrum. Treated with fuming (yellow) nitric acid it gives Gmelin's reaction, starting now with a blue colour as a product of the first stage of its oxidation. It also yields Huppert's reaction. (See above sub bilirubin.)

Like bilirubin the quantitative determination of biliverdin is dependent upon spectrophotometric methods.[3]

The formula assigned above to biliverdin represents its formation from bilirubin by simple oxidation.[4] This is undoubtedly correct as against the older view of Staedeler that the change consists not only in the assumption of oxygen but also of a molecule of water.

Bilifuscin, bilihumin, and biliprasin are the names given by Staedeler to ill-defined and probably impure products obtained during his investigations on bile-pigments as obtained from gall-stones. Biliprasin is apparently only impure biliverdin (Maly).

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[1] Maly, *Sitzb. d. k. Akad. Wien*, Bd. LXX. 3 Abth. 1874. Juli-Hft.
[2] Maly, *Ibid.* Bd. LXXII. 3 Abth. 1875. Oct.-Hft.
[3] See references sub bilirubin.
[4] Maly, *loc. cit.* Thudichum, *Jl. Chem. Soc.* July, 1876.

3. **Bilicyanin.**[1] (Cholecyanin, Choleverdin.)

This is the substance which results from the oxidation of biliverdin and is the cause of the blue colour observed when bile is treated with fuming (yellow) nitric acid as in Gmelin's reaction. It has not as yet been isolated either in sufficient quantity, and still less in a condition of sufficient purity, to admit of such a chemical investigation as would lead to the determination of its composition. But by analogy with the known relationship of biliverdin to bilirubin, and from the evidence afforded by the composition of choletelin (see below) into which bilicyanin may be readily converted by further oxidation, bilicyanin will probably be found to differ from biliverdin simply by the addition of oxygen to the molecule of the latter.

Preparation. Bilirubin is dissolved in chloroform or suspended in alcohol and slowly oxidised either by gradual addition of bromine or fuming nitric acid; as soon as the mixture is of a bright blue colour, the bilicyanin is precipitated by an excess of water. As thus obtained it is insoluble in water, almost insoluble in either ether or chloroform, but soluble in alcohol and alkalis. In presence of alkalis it is still almost insoluble in either ether or chloroform; in presence of acids it is now scarcely soluble in water, but soluble in ether and chloroform.

Bilicyanin is for practical purposes characterised solely by its marked absorption spectrum. This consists of three bands,— one on each side of *D*, that to the red side of *D* being the darkest, and one between *b* and *F*. The latter is probably identical with the band seen in acid solutions of choletelin and due to the production of this substance in small quantity during the oxidation of bilirubin. The position of the bands varies somewhat according to the solvent employed and as to whether the solution is acid or alkaline.

During the application of Gmelin's test for bile-pigments the blue due to bilicyanin is bordered by a violet colour and this by a red, the final and permanent colour being yellow. Of these three the first is not as yet known to be definitely due to one specific substance; it is most probably the result of a mixture of the blue of bilicyanin with the red of the next product. The red colour is on the other hand supposedly due to a definite pigment sometimes called bilipurpurin, of which however nothing definite is as yet known. The yellow marks the final formation of choletelin.

4. **Choletelin.** $C_{16}H_{18}N_2O_6$. (?)

This is the final product of the oxidation of bile-pigments. It is readily obtained by suspending bilirubin in alcohol and oxidising it by passing the fumes of nitrous acid into the mixture. As soon as the play of colours is complete and the solution is of a pure yellow colour, it is poured into a large excess of water, from which on more or less prolonged standing choletelin separates out as a flocculent mass, which if washed and dried yields a brown powder.[1] It is readily soluble in alkalis, as also in either alcohol, chloroform, or ether, but least so in the two last solvents. None of the solutions exhibit any fluorescence even after the addition of zinc chloride. In this it differs markedly from urobilin, a well-known yellow urinary pigment. The above statements scarcely provide any certain means of identifying choletelin as a chemical substance, and no specific test for it has as yet been described. Neither is it quite certainly characterised by its absorption spectrum, so far at least as any specific bands are concerned. Indeed there has been very great difference of opinion as to whether it ever gives any band at all, and if it does, where this band is situated. With our existing knowledge it seems safe to say that in alkaline solutions choletelin shows no absorption band, and that in acid solutions a band may be, and frequently is seen, lying between *b* and *F*. The uncertainty as to its spectroscopic properties led some of the older observers [2] to regard choletelin as identical with hydrobilirubin (urobilin). This view is however quite untenable both as the result of purely chemical investigations [3] and of spectrophotometric determinations of the optical properties of the two substances.[4]

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[1] Heynsius u. Campbell, Pflüger's *Arch.* Bd. IV. (1871), S. 526, X. 1875, S. 246, gives literature of this and other bile-pigments.

5. **Hydrobilirubin.** $C_{32}H_{40}N_4O_7$.

When bilirubin is dissolved in dilute caustic potash or soda or suspended in water and treated with sodium-amalgam in successive portions, air being at the same time carefully excluded, it is observed that at first no hydrogen is evolved; the dark-coloured solution becomes gradually lighter in colour and more transparent, until at the end of two or three days it is bright yellow or brownish-yellow, and now hydrogen begins to come off from the mixture. At this stage the supernatant fluid should be poured off from the metallic mercury which has accumulated, and if it is now acidulated strongly with either hydrochloric or acetic acid, it yields a more or less copious flocculent precipitate of a dark reddish-brown colour. This precipitate is impure hydrobilirubin. It is purified by being redissolved in ammonia, reprecipitated from this solution by the addition of acid, and finally washed with water. At first during the washing a considerable amount of the substance passes into solution, but as the merely adherent salts are washed away, it becomes less and less soluble in water until at last it is almost insoluble. When dried it takes the form of a dark reddish-brown amorphous powder, which is readily soluble in alcohol and chloroform, and but sparingly soluble in pure ether. It is also very soluble in alkaline solutions, to which it imparts a yellow colour as of normal urine: when acidulated the solutions turn red.[1]

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[1] Maly, *Sitz. d. k. Akad. d. Wiss. Wien*, Bd. LVII. (1868), 2 Abth. Feb.-Hft. LIX. (1869), 2 Abth. Ap.-Hft.

[2] Heynsius u. Campbell, *loc. cit.* Stokvis, *Centralb. f. d. med. Wiss.* 1873, S. 211, 449.

[3] Maly, *Ibid.* S. 321, and more particularly Liebermann, Pflüger's *Arch.* Bd. XI. (1875), S. 181.

[4] Vierordt, *Zt. f. Biol.* Bd. X. (1874), S. 399.

The acid solutions of hydrobilirubin show a marked absorption band between *b* and *F* which becomes fainter if ammonia is added until the reaction is alkaline. But on the subsequent addition of a few drops of a solution of zinc chloride, the band reappears with usually increased intensity, though shifted slightly towards the violet end of the spectrum.[2] This alkaline solution to which the zinc salt has been added also shows, in marked distinction to the acid solutions, a brilliant fluorescence which is most characteristic of the substance, being of a bright rosy-red colour by transmitted, and bright green by reflected light.

Previously to the discovery of hydrobilirubin by Maly, a well-characterised urinary pigment had been isolated and described by Jaffé under the name of urobilin (see below), while about the same time that Maly's work was carried on, a pigment had been obtained from fæces and described, under the name of stercobilin, as identical with urobilin.[3] Careful comparison by Maly of his hydrobilirubin with urobilin led him to assert the complete identity of the two substances. This view has been most generally adopted, and is probably correct as a broad statement of facts. There are on the other hand several observers who have expressed themselves against the exact identity of these substances.[4] Their views are however based on comparatively slight and inconclusive spectroscopic differences between the natural and artificially prepared substances and on other differences, such as of the intensity of their fluorescent activity, which are still less conclusive. For the present the evidence of close relationship if not of absolute identity suffices fully as a basis for our belief in the genetic relationship of the bile and urinary pigments and of the ultimate derivation of these from the colouring-matter of the blood.

During his earlier researches on the pigments of blood Hoppe-Seyler described a product resulting from the reduction of hæmatin in acid solution by the action of zinc and hydrochloric acid, characterised by one absorption band between *b* and *F* and, as he then said, two other bands.[1] After the appearance of Maly's work he was led to suspect that the substance he had previously described was in reality identical with hydrobilirubin and therefore with urobilin, a conclusion which he verified by a careful repetition of his earlier experiments.[2]

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[1] Maly, *Centralb. f. d. med. Wiss.* 1871, S. 849. *Annal. d. Chem.* Bd. 163 (1872) S. 77.

[2] Vierordt, *Zt. f. Biol.* Bd. IX. (1873), S. 160. See later 'Quantitative Spectralanalyse,' 1876, S. 99.

[3] Vanlair u. Masius, *Centralb. f. d. med. Wiss.* 1871, S. 369. Cf. Jaffé, *Ibid.* S. 465.

[4] See MacMunn, *Clinical Chemistry of Urine*, 1889, p. 105, or *Jl. of Physiol.* Vol. x. (1889), p. 72. Contains all necessary references. But as against Disqué see also Maly, Pflüger's *Arch.* Bd. xx. (1879), S. 331.

More recently Nencki and Sieber have prepared a similar pigment by the action of hydrochloric acid and zinc on their hæmatoporphyrin, to which latter substance, as was stated above, they assigned a formula identical with that of bilirubin. They state however that the pigment (urobilin) is not quite identical as obtained on the one hand by the action of nascent hydrogen on bilirubin, and on the other hand on their hæmatoporphyrin.[3]

Assuming then the identity of these substances we have in Hoppe-Seyler's work the best and most direct chemical evidence of the relationship between the colouring-matters of the blood and bile. For if one and the same substance, viz. urobilin, can be prepared by the same means, namely reduction (hydrogenation) from both hæmatin (hæmoglobin) and bilirubin, these two substances must be themselves closely related. It has not however as yet been found possible to produce a bile-pigment directly from hæmoglobin or hæmatin by any artificial process outside the animal body. The derivation of the urinary pigments (urobilin) from those of bile presents no difficulty when it is remembered that a not inconsiderable quantity of hydrogen is present in the gases of the intestine (§ 282) which may be accounted for by (butyric) fermentative processes (p. 105), and that this hydrogen might in its nascent state readily produce the simple change which is known to occur when bilirubin is converted into hydrobilirubin or urobilin. And here it is interesting to note that hydrobilirubin is readily absorbed and excreted in the urine either when placed in the alimentary canal or injected subcutaneously.

The question of pigmentary relationships to which reference has just been made suggest the present as a convenient place to enter into further details on the now undoubted but once disputed derivation of the bile-pigments from the colouring-matter of blood (see § 477).

The starting point for this view was the discovery and description of hæmatoidin crystals by Virchow (see p. 239) as occurring in old blood-clots in parts of the body remote from the liver and in which it was inconceivable that they could have arisen by any process other than a gradual formation from the pigment of the red corpuscles, followed as this was by proofs of the identity of hæmatoidin and bilirubin. This was followed[1] by experiments on the injection of bile-salts into the blood and an accompanying output of bile-pigments in the urine, to which the true significance was subsequently attached by Kühne, namely that the pigments arose from a conversion of hæmoglobin set free from the corpuscles under the solvent action of the bile-salts. This he confirmed by injections of hæmoglobin in solution.[2] These views were however opposed on the basis of similar experiments in which it was stated that either no bile-pigments appeared in the urine as the result of injections of hæmoglobin into the vascular system, or that if they did, they were due merely to an accumulation of that small amount which is frequently present in the urine of dogs.[3] But the careful subsequent experiments of Tarchanoff, in which he endeavoured to avoid many obvious sources of error present in those of Naunyn and Steiner, are more usually regarded as having afforded definite and conclusive confirmation of the earlier views.[4] This observer further found a considerably increased amount of bile-pigments in the bile collected during the experiments, and came to the conclusion that the conversion of blood- into bile-pigments takes place in the blood-vessels, a part being excreted in the urine, while the larger part passes out in the bile. He showed in confirmation of earlier experiments [5] that the liver is extremely active in excreting bilirubin injected into the blood-vessels; practically the whole of it passes out in the bile.[6] The relationships thus indicated receive further confirmation from the observation that in many pathological conditions of the horse, bile-pigments are copiously found in its tissues and transudations, accompanied by blood-pigments, and that solutions of hæmoglobin when injected into the subcutaneous tissue of this animal become after a few days partially converted *in situ* into granules and flakes which are of a yellow or orange colour and yield an intense Gmelin's reaction.[7] Finally by the action of phenylhydrazin on hæmatin and on bilirubin products are obtained which in each case exhibit a similar and marked play of colours under the action of fuming (yellow) nitric acid.[8]

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[1] *Med.-chem. Untersuch.* Hft. 4, 1871, S. 536.

[2] *Ber. d. d. chem. Gesell.* Bd. VII. (1874), S. 1065.

[3] *Monatsh. f. Chem.* Bd. IX. (1888), S. 115; *Arch. f. exp. Path. u. Pharmakol.* Bd. XXIV. (1888), S. 430.

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[1] Frerichs u. Staedeler, Müller's *Arch.* Jahrg. 1856, S. 55.

[2] Virchow's *Arch.* Bd. xiv. (1858), S. 310. Cf. *Physiol. Chem.* 1868, S. 89.

[3] Naunyn, *Arch. f. Anat. u. Physiol.* Jahrg. 1868, S. 401. Steiner, *Ibid.* 1873, S. 160. Contain full references to all then existing literature.

[4] Pflüger's *Arch.* Bd. ix. (1874), Sn. 53, 329.

[5] Feltz et Ritter, *Jn. de l'Anat. et de la Physiol.* 1870, p. 315. Cf. Vossius, *Arch. f. exp. Path. u. Pharmakol.* Bd. xi. (1879), S. 426.

[6] See later Stadelmann, *Ibid.* Bd. xv. (1882), S. 237, and (in connection with the next reference) Bd. xxvii. (1890), S. 93.

[7] Latschenberger, *Zt. f. Veterinärkunde*, Bd. i. (1886), S. 47. *Monatsh. f. Chem.* Bd. ix. (1888), S. 52.

[8] Filehne, *Verhand. d. Congresses f. inn. Med. Wiesbaden*, Ref. in *Centralb. f. klin. Med.* 1888.

One point still remains for discussion. It has been seen that bile-pigments can be formed from those of the blood in outlying parts of the body without the intervention of the liver. Are we therefore to suppose that the liver is similarly inoperative in that increased formation and excretion of bile-pigments, both in the urine and bile, which result from the intravascular injection of hæmoglobin? Opinions have differed on this point. It is on the whole more probable that the liver is in all cases the chief factor in the conversion. The *normal* production of bile-pigments is entirely due to hepatic activity, for no pigments are accumulated in the body after extirpation of the liver in frogs or its exclusion from the circulation in birds.[1] This accords with the fact that apparently the larger part of the pigments resulting from the injection of hæmoglobin pass out in the bile while but little goes into the urine. If this is so, how shall we account for the excretion of the latter and smaller portion by the kidneys? It is known that the liver is peculiarly liable under the influence of but slight operative and other influences to pass some of its products over into its lymphatics whence they make their way into the blood-vessels and may hence be excreted by the kidneys. Very slight obstruction of the bile-duct suffices to produce this result, and it has been observed that the bile formed after injections of hæmoglobin is unusually viscid. The views here put forward (see also § 477) are further in complete accord with the facts that hæmatin (hæmochromogen) readily loses iron and yields hæmatoporphyrin $C_{32}H_{32}N_4O_5$ which differs but slightly in composition from bilirubin $(C_{16}H_{18}N_2O_3)_2$, and that it is precisely in bile and very largely in the liver that we meet with considerable quantities of iron in some as yet not well-known form.[2] The possible function of the spleen as an organ in which a considerable disintegration of red corpuscles takes place, in providing the material requisite for the formation of bile-pigments by the liver has been already discussed[3] (§ 478).

As already stated herbivorous bile, as of ox and sheep, frequently shows absorption bands even when fresh. These are regarded by MacMunn as due to a substance to which he has given the name cholo-hæmatin since it occurs in bile and, as the action of sodium-amalgam shows, is related to hæmatin. The bands more usually seen are three, two near *D* and one near *E*.[4]

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[1] Stern, *Arch. f. exp. Path. u. Pharm.* Bd. XIX. (1885), S. 39. Minkowski u. Naunyn, *Ibid.* Bd. XXI. (1886), S. 1.

[2] Zaleski, *Zt. f. physiol. Chem.* Bd. X. (1886), S. 453. See also Virchow's *Arch.* Bd. CIV. (1886), S. 91.

[3] According to Schäfer, *Proc. Physiol. Soc.* 1890, No. 3 (see *Jl. of Physiol.* Vol. XI.), there is no evidence of any discharge of hæmoglobin from the spleen in the blood of the vein of this organ.

[4] *Jl. of Physiol.* Vol. VI. (1884), p. 24.

THE PIGMENTS OF URINE.[1]

When fresh normal urines are examined spectrophotometrically it is found that the extinction coefficients (see p. 225) for any given portion of the spectrum of the several fluids do not bear a constant ratio each to the other. If the urines contained only one colouring-substance, then no matter how much the absolute value of the extinction coefficients varied for different regions of the spectrum, their ratios would be constant for any given region. From this it appears probable at the outset that even normal urine is coloured by at least two if not more pigments.[2] Our knowledge of these pigments is at present imperfect and almost limited to that of one substance, namely urobilin, and even with respect to this one, considerable difference of opinion exists as to its nature and relationships to the other pigments of the body from which it is supposed to be ultimately derived. The reasons for this are simple. It is extremely probable that normal urine is often coloured by some chromogenic mother-substance (cf. zymogens) rather than by the fully formed pigment. In the next place, since the colouring-matters are normally present in but very small amount, and since they are not known to be crystallisable or to form definite compounds with well-known precipitants, they have not as yet, with the exception perhaps of urobilin, been obtained either with any guarantee of their purity or in quantities sufficient to admit of ultimate analysis. Hence our knowledge of them is chiefly based upon their spectroscopic properties. They are further most probably far from stable substances, so that they may undergo some considerable change either by mere exposure to the air (oxygen) or as the result of the various and often different methods of extraction and preparation employed by various authors. This, together with the fact that the position of the absorption bands may vary somewhat with the reaction of the solution and the nature of the solvent, &c., accounts with but little doubt not only for the extremely numerous and insufficiently characterised pigments which have at one time or another been obtained from urine, but also for much of the conflict and confusion of opinion which exists as to the nature and relationships of those pigments of which we can speak with most confidence.

1. **Urobilin.** $C_{32}H_{40}N_4O_7$. (?)

This, the best known and most definitely characterised of the urinary pigments, was first described by Jaffé who regarded it as the chief colouring-substance of normal urine, while present in much larger amounts in the urine of fever.[1] He also obtained it occasionally from bile, the name urobilin thus indicating its double source. In fresh normal urine the amount was frequently extremely small, but was observed to increase on standing exposed to the air (oxygen), a result due to the probable presence in the urine of some chromogen or mother-substance (urobilinogen)[2] of the urobilin. The amount of this pigment in urine is too small to provide adequate material for an elementary analysis, so that it was at first characterised by its solubilities in various fluids, by the strongly-marked fluorescence of certain of these solutions and more particularly by the absorption-spectrum it exhibited. The subsequent preparation of hydrobilirubin from bilirubin, and the establishment of its identity with urobilin (p. 246) provided for the first time a mass of the substance sufficient to admit of analysis, and upon this the formula given above for urobilin is based. It must not however be forgotten that the identity of the two pigments is disputed by several observers, although the balance of belief seems as yet to support it. It will conduce to clearness if we incline for the present to this belief and describe the preparation and properties of urobilin as given by Jaffé, on the assumption that it is identical with hydrobilirubin, and then subsequently give a short account of the opposing views.

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[1] For references to the principal earlier works on urinary pigments see Udránszky, *Zt. f. physiol. Chem.* Bd. XI. (1887), S. 537, and for all details consult Neubauer u. Vogel, *Analyse des Harns*, Aufl. IX. 1890.

[2] Vierordt, *Die quantit. Spectralanalyse,* 1876, S. 78.

Preparation from urine. Several methods may be adopted; of these only the broader facts can here be given, but they suffice to provide solutions which exhibit the characteristic spectra. (i) When urine contained much urobilin Jaffé precipitated it by the addition of chloride of zinc in presence of an excess of ammonia; if but little, then by the addition of basic lead acetate. These precipitates were then worked up by processes which do not admit of a suitably brief description.[3] (ii) Precipitate the urine completely by the addition first of normal lead acetate, then of the basic acetate. Wash the precipitates, dry at low temperature, and extract with absolute alcohol (not methylated spirit) acidulated with 1 — 2 p.c. of sulphuric acid. This extract may be then diluted with water and the pigment extracted by shaking up with chloroform, in which it is readily soluble.[4] (iii) The urine is acidulated with 0·2 p.c. of sulphuric acid and then saturated with neutral ammonium sulphate. The precipitate thus obtained is then collected on a filter, washed with an acidulated saturated solution of the ammonium salt, freed by pressure from adhering fluid, and dissolved by gentle warming in absolute alcohol to which if necessary a few drops of ammonia have been added.[1] (iv) Frequently from normal urine, the more readily if that be highly coloured, a solution of urobilin may be obtained by simple agitation with chloroform, or by gently shaking it up with half its volume of *pure* ether free from all traces of alcohol. The ether is then removed by a separating funnel, evaporated at ordinary temperatures, and the residue dissolved in a small quantity of absolute alcohol.[2]

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[1] *Centralb. f. d. med. Wiss.* 1868, S. 243; 1869, S. 177. Virchow's *Arch.* Bd. XLVII. (1869), S. 405.

[2] For further references see Neubauer u. Vogel, *Anal. d. Harns.* 1890, Sn. 331, 336.

[3] For details see Neubauer u. Vogel, *loc. cit.* S. 334.

[4] Mac Munn, *Proc. Roy. Soc.* pp. 26, 206. *Jl. of Physiol.* Vol. x. (1889), p. 71.

If the alcoholic or chloroformic solutions above described are evaporated to dryness at a low temperature, the urobilin remains as a yellowish-brown amorphous pigment, which is practically insoluble in water except in presence of small amounts of neutral salts, very slightly soluble in either ether or benzol, readily soluble in alcohol and in chloroform. The neutral alcoholic solutions if dilute are yellow with a rosy tint, and if strong show a green fluorescence. The acid solutions are reddish-yellow, or if dilute bright rose-coloured and do not fluoresce. Alkaline (alcoholic) solutions are yellow or yellowish-green according to the concentration and usually show a marked fluorescence, which is much increased on the addition of a solution of zinc chloride, appearing now rose-coloured by transmitted light and brilliant green by reflected.

Spectra of urobilin. Neutral or alkaline alcoholic solutions show one absorption band between *b* and *F*. In alkaline solution the band is frequently very faint, but is more strongly marked after the addition of zinc chloride, so much so that it can often only be distinctly seen after the addition of this salt. In acid solutions a similar band is seen, situated however in this case slightly more towards the violet end of the spectrum.

The methods given above for the preparation of urobilin, indicate sufficiently the procedure requisite for its detection in solutions. As already stated (p. 246) the position of the absorption band of urobilin is very similarly situated to that of choletelin under certain conditions. The conflict of opinion as to the identity of the two substances has been dealt with above.

It now remains to give a short account of the more recent views on urobilin and its relationship to other pigmentary substances to which reference has already been made.[3]

Mac Munn distinguishes between two forms of urobilin, viz. normal and febrile or pathological. They are both obtained from urine by the same method (see above) and differ as follows.

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[1] Méhu, *Journ. d. pharm. et de chim.* T. XXVIII. (1878), p. 159. This method is good, as avoiding to a considerable extent any alteration of the pigments by the process employed.

[2] E. Salkowski, *Zt. f. physiol. Chem.* Bd. IV. (1880), S. 134.

[3] Mac Munn, *Clinical Chem. of Urine*, 1889, p. 104. Gives all necessary references. For spectra see *Jl. of Physiol.* Vol. X. (1889), p. 116.

(i) *Normal urobilin.* In acid alcoholic solution it shows one absorption band, close to and enclosing *F*: this band disappears when the solution is neutralised by alkalis. If treated with zinc chloride in presence of ammonia this band is replaced by one narrower and nearer the red end of the spectrum, while at the same time a green fluorescence is observed, but much less marked than in the case of febrile urobilin. (ii) *Febrile urobilin.* The solubilities of this substance are the same as of the preceding form. On the other hand the band at *F* is broader and darker than is that of normal urobilin, and further in an *ethereal* acid solution two other bands may be seen, one adjoining *D* towards the red, the other mid-way between *D* and *E*. These last two bands are invisible in urine. By prolonged action of sodium amalgam on an alcoholic solution of normal urobilin, fibrile urobilin is obtained. The spectrum of normal urobilin is the same as that of choletelin, but the substances differ with respect to the greater ease with which choletelin may be reduced to febrile urobilin. Normal urobilin is regarded as differing from hydrobilirubin, the evidence being deduced from spectroscopic observations. Febrile urobilin on the other hand is identical with stercobilin and is apparently the pigment to which the absorption spectra of the bile of some animals is due.[1]

In concluding this account of urobilin and allied substances it may be well once more to draw attention to the fact that the differences of opinion among the various observers is based almost entirely on spectroscopic appearances. These are far from conclusive for there is no guarantee that in any given case the solution under examination contains only *one* pigment. It may contain at most a preponderance of this one but frequently mixed with other pigments which are derived either from the fluid originally operated upon, or are decomposition products resulting from the action of the reagents employed.[2] The final solution of the questions raised above will only be supplied by a purely chemical investigation of the several substances under discussion; such an investigation would however be one of extreme difficulty.

Thudichum considered that normal urine contains only one pigment, which he called urochrome.[3] Maly regarded this as the same as urobilin.[4] More recently Thudichum has upheld his former views.[5]

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[1] Mac Munn, *The Spectroscope in Med.* 1880, p. 156. A tabular conspect of the above statements is given by Halliburton, *Chem. Physiol. and Pathol.* 1891, p. 752.

[2] Thus Vierordt has shown that if the urinary pigments are precipitated by the acetates of lead and extracted from this by absolute alcohol acidulated with oxalic acid, the coloured solution thus obtained possesses optical properties quite different from those of the original urine; a result which indicates that the pigments have been considerably changed during extraction. *Die quantitat. Spectralanalyse*, 1876, S. 96.

[3] *Brit. Med. Jl.* No. 201, 1864, p. 509.

[4] Liebig's *Ann.* Bd. CLXIII. (1872), S. 90.

[5] *Jl. Chem. Soc.* Ser. 2, Vol. XIII. (1875), pp. 397, 401.

2. **Uroerythrin.**

This is a pigment of which but little is known. It is regarded as the colouring-substance of certain bright-red (pink) urinary deposits and as possibly occurring in the highly coloured urines of rheumatism, &c. It appears to be an amorphous reddish substance, with an acid reaction, slowly soluble in either water, alcohol, or ether.[1] Treated with caustic alkalis it turns green, more particularly when in the solid form. In alcoholic solution obtained by boiling pink urates with alcohol it shows two ill-defined absorption bands between *D* and *F*.[2]

3. **Urohæmatoporphyrin.**

This pigment was first described by Mac Munn (under the name of urohæmatin) as occasionally occurring in certain pathological urines as of acute rheumatism, Addison's disease, &c. and to it he gave the present name from certain resemblances of its spectra to those of hæmatoporphyrin.[3] It is obtained from urine by the method employed for the separation of urobilin, or artificially by the action of reducing agents on hæmatin, this being the supposed source of its origin in the body. It is soluble in either alcohol, ether, benzol, or chloroform. In acid alcoholic solution it shows three absorption bands, one narrow adjoining *D* on the red side of this line, one half way between *D* and *E*, and one between *b* and *F* closely resembling the band of urobilin. There is also occasionally a fourth very faint band between the first two bands described above. In alcoholic solution made alkaline by ammonia it yields a spectrum closely resembling that of hæmatoporphyrin (see above p. 238). But unlike the latter substance its solutions show a very faint green fluorescence on the addition of zinc chloride and ammonia. The occurrence of hæmatoporphyrin in urine has been frequently recorded[4] and from the spectroscopic appearances described above, some observers are inclined to the view that urohæmatoporphyrin is not a single substance but a mixture of hæmatoporphyrin with some pigment closely resembling urobilin.

Urohæmatoporphyrin is perhaps closely related to two pigments known as urorubrohæmatin and urofuscohæmatin obtained from a case of leprosy[5] (Mac Munn).

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[1] Heller, in his *Archiv.* (2) Bd. III. (1854), S. 361.

[2] Mac Munn, *Proc. Roy. Soc.* Vol. XXXV. (1883), pp. 132, 370.

[3] *Jl. of Physiol.* Vols. VI. (1884), p. 36; X. (1889), p. 73.

[4] See most recently E. Salkowski, *Zt. f. physiol. Chem.* Bd. XV. (1891), S. 286. There was in the cases examined some evidence that the occurrence of hæmatoporphyrin in the urine was perhaps not unconnected with the administration of sulphonal.

[5] Baumstark, *Ber. d. d. chem. Gesell.* Bd. VII. (1874), S. 1170. Pflüger's *Arch.* Bd. IX. (1874), S. 568. Cf. Hoppe-Seyler, *Physiol. Chem.* 1879, S. 875.

4. **Humus pigments.**

When carbohydrates are treated with acids or alkalis, among the numerous products which arise are certain pigmentary bodies of a more or less dark-brown colour. A similar colouration is well known as occurring in fruits when bruised or exposed to the air,[1] and generally in decaying vegetable tissues. These substances are known under the name of 'humus.' When urine is treated with acids in presence of oxygen it acquires a markedly darker colour, and since carbohydrates in small amount are probably present in all urines,[2] there is at once a possibility that some at least of the observed colouration is due to the production of humus-pigmented substances by the action of the acids on the carbohydrates. In accordance with this view certain so-called humus pigments have been prepared from urine, but our knowledge of them is as yet very incomplete. They are stated to be practically insoluble in any solvents other than amyl-alcohol, strong ammonia, and caustic alkalis: the solutions show no absorption bands when examined spectroscopically. They are further said to account for the usually dark colour of normal herbivorous urine and of urine after the cutaneous absorption of carbolic acid and several other aromatic compounds.[3]

It is very probable that several dark-coloured pigments such as the uromelanins of Plósz and Thudichum obtained by the action of acids on urinary pigments or chromogens are allied to if not identical with these humus substances.

5. **Urinary melanin.**[4]

Certain tumours are not infrequently observed which from their extremely dark pigmentation are spoken of as 'melanotic,' the colouring-substance being known as melanin.[5] The urine of patients suffering from these tumours is either dark-brown or black when voided, or speedily assumes this colour after brief exposure to the air or by the action of nitric acid or other oxidising agents, the pigment to which the colour is due being apparently identical with that present in the tumour. This action of oxidising agents indicates that here also, as in the case of other urinary pigments, there is primarily some chromogenic forerunner (melanogen) of the actual pigment. This chromogen may be partially precipitated from the urine by baryta water and completely by normal lead acetate. When the latter precipitate is suspended in water and decomposed by sulphuretted hydrogen, it yields a colourless solution which when evaporated to dryness leaves a dark amorphous residue insoluble in water, ether, cold alcohol, acetic acid, and dilute mineral acids. The fully formed pigment may, like its chromogenic forerunner, be partially precipitated by baryta water, the remainder being precipitable by the subsequent addition of normal lead acetate. The baryta precipitate contains the larger amount of the pigment, and from it the colouring-matter may be more easily obtained than from the precipitate with the lead salt, since the latter carries down other urinary pigments at the same time. The isolation of the urinary melanin in a pure form from the baryta compound admits of no suitably concise description; it must suffice here to state that an impure product is obtained by decomposing the compound with sodium carbonate assisted by gentle warmth and precipitating the pigment from the resulting solution by a slight excess of sulphuric acid. The product when purified is partly insoluble, partly soluble in acetic acid of 50—75 p. c. Of these portions the former when dried is a brownish-black amorphous powder, insoluble in either water, alcohol, ether, chloroform, or dilute (mineral) acids, but readily soluble in alkalis. The latter was obtained in too small amounts to admit of complete investigation. On analysis the pigment was found to contain iron (·2 p. c.) and a considerable amount of sulphur (9 p. c.) and not to show any absorption bands when its solutions were examined spectroscopically.

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[1] Hoppe-Seyler, *Zt. f. physiol. Chem.* Bd. XIII. (1889), S. 66.

[2] See Wedenski, *Ibid.* S. 122. E. Salkowski, *Ibid.* S. 270.

[3] Udránszky, *Ibid.* Bde. XI. (1887), S. 537, XII. (1888), S. 33. Contains very full references to other works.

[4] Mörner, *Zt. f. physiol. Chem.* Bde. XI. (1887), S. 66, XII. (1888), S. 229. Gives list of literature to date. See also Zeller, Langenbeck's *Arch.* Bd. XXIX. (1884), S. 2, and later Brandl u. Pfeiffer, *Zeitsch. f. Biol.* Bd. XXVI. (1890), S. 348.

[5] The name melanin is more usually applied as a generic title for the dark-brown or black pigments such as occur in the hair, epidermis, retinal epithelium, choroid, &c.

This pigment appears to be identical with one previously described under the name of phymatorhusin as obtained from melanotic tumours, and closely allied to hyppomelanin obtained from similar tumours of the horse.[1]

When melanotic urines are treated with solutions of ferric chloride, they yield, according to the concentration of the reagent, either a dark-brown cloudiness or else a black precipitate soluble in excess of the precipitant: this test is both delicate and characteristic. Further when to these urines a dilute solution of sodium nitroprusside and some caustic potash is added they frequently show a pink or red colouration which turns blue on the addition of acids, owing to the formation of Prussian blue. The latter reaction is not due to the melanotic pigment but to some other substance simultaneously excreted.[2]

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[1] Berdez u. Nencki, *Arch. f. exp. Pathol. u. Pharmakol.* Bd. xx. (1886), S. 346. Nencki u. Sieber, *Ibid.* Bd. xxiv. (1888), S. 17. See also Miura, Virchow's *Arch.* Bd. cvii. (1887), S. 250.

[2] v. Jaksch, *Zt. f. physiol. Chem.* Bd. xiii. (1889), S. 385.

6. Indoxyl-pigments.

Of the total indol formed in the alimentary canal, a portion is excreted with the fæces, while the remainder is absorbed and reappears in the urine united with potassium as ethereal compounds of indoxyl with either glycuronic acid (p. 107) or sulphuric acid (p. 199), the latter being known as urinary indican. When warmed with hydrochloric acid these compounds are decomposed, yielding indoxyl and the potassium salt of the corresponding acid. If the decomposition is effected in the absence of oxygen, the indoxyl may be in part gradually changed into an amorphous reddish substance, indigo-red, which is insoluble in water, but yields a red solution when dissolved in alcohol, ether, or chloroform.[1] These solutions show no certainly characteristic absorption bands. In presence of oxygen and with most certainty by the action of an oxidising agent, the indoxyl is readily converted into indigo-blue, whose properties and solubilities have been already sufficiently described. Dilute solutions of indigo-blue exhibit in thin layers one absorption band in the red lying between *a* and *B* 25 *C*; if the thickness of the solution be increased this band widens out towards *D* and at the same time a second faint band makes its appearance in the green lying between *D* 50 *E* and *D* 77 *E*.[2]

The numbers just given refer to the method (Vierordt's) frequently used for indicating the position of an absorption band. In this the distance between any two of the fixed lines of the solar spectrum is regarded as being divided into 100 equal parts and the extent of the band is given by reference to these divisions. Thus if a band is described as lying between *D* 50 *E* and *D* 77 *E* it implies that the band begins half way ($\frac{50}{100}$ of the distance) between *D* and *E* and extends to $\frac{77}{100}$ of the distance between the same two lines.[3] (See also above, note 1, p. 228.)

Variable accounts of the above pigments may be obtained from urines during their spontaneous decomposition or when treated with hydrochloric acid or oxidising agents, the amount being greatest in herbivorous urine and especially great in certain pathological urines (see p. 199). They have also been met with in urinary sediments and calculi.[4]

7. Skatoxyl-pigments.

The skatol formed in the alimentary canal gives rise, like indol, to compounds of skatoxyl with either sulphuric acid or glycuronic acid (see p. 202). These compounds when decomposed by hydrochloric acid or oxidising agents give rise to a colouring-matter which is more or less red and may exhibit a distinct and strong purple tint.[1] The pigment is insoluble in water, but soluble in either alcohol or chloroform, also when freshly prepared in ether but less so if it has been kept some time. Alcoholic solutions are of a reddish-violet colour; ethereal solutions may show a green fluorescence, which on exposure to the air takes on a reddish tinge. It is also soluble in hydrochloric and sulphuric acids, giving bright red or pink solutions, and in alkalis yielding yellow solutions. No absorption bands for this substance have as yet been described and the whole subject requires further investigation.

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[1] Cf. Nencki, *Ber. d. d. chem. Gesell.* Bd. IX. (1876), S. 299, and see Mac Munn, *Proc. Roy. Soc.* Vol. XXXV. (1883), p. 370.

[2] Vierordt, *Zt. f. Biol.* Bde X. (1874), S. 27, XI. (1875), S. 192.

[3] A table for the conversion of these data into wave-length limits is given by G. u. H. Krüss, *Kolorimetrie u. quant. Spektralanalyse*, 1891, S. 290.

[4] Ord, *Berl. klin. Wochensch.* 1878, S. 365. Chiari, *Prager med. Wochensch.* 1888, S. 541.

A considerable number of red or reddish-purple pigments have at different times been obtained and described under specific names as derived either from pathological urines when first voided, or from the spontaneous decompositions of or action of mineral acids on different urines. The remarks which have been made on the indoxyl and skatoxyl pigments indicate a possibility that they may all have a common origin and thus be closely related if not in many cases identical. In the absence of any guarantee of the purity of the several coloured products and of their not having undergone some change during the operations involved in their preparation, no authoritative statement on this point can as yet be made. Indeed the whole subject of the origin, nature, and relationships of urinary pigments is at present in a state of considerable confusion and uncertainty.[2]

The urinary pigments so far dealt with may be regarded as either normal or pathological, or as resulting from the spontaneous or artificial decomposition of urinary constituents which are at the outset colourless. In addition to these, other colouring substances are not infrequently observed, or colour-reactions obtained, in urines passed after the administration of certain drugs or the consumption of certain vegetable tissues. They are in many cases not unimportant as leading at first sight to possibly erroneous conclusions as to the presence in urine of pathologically important pigments, *e.g.* of bile or blood. After the administration of rhubarb or senna, the urine may be yellow or greenish-yellow, due to the presence of chrysophanic acid [$C_{14}H_5(CH_3)(OH)_2O_2$], and similarly after the use of santonin ($C_{15}H_{18}O_3$). In such cases if the urine is strongly alkaline it may be of a red colour; this is changed to yellow on the addition of hydrochloric acid, and if it is initially acid, it turns red on the addition of an excess of alkali.[1] After the internal administration of copaiba, the urine turns pink or rose-coloured on the addition of hydrochloric acid and shows three absorption bands, one (narrow) in the orange to the red side of *D*, one broad band in the green between *D* and *E*, similar to that of fuchsin, and one in the blue.[2] Tannin leads to the appearance in urine of gallic acid [$C_6H_2 . (OH)_3 . COOH$], which is hence sometimes found normally in the urine of herbivora (horse).[3] In such cases the urine if made alkaline with caustic potash turns brown, and bluish-black on the addition of ferric chloride. It also yields a pink colouration with Millon's reagent, similar to that given by proteids or tyrosin. After doses of antipyrin [$C_9H_6N_2O(CH_3)_2$] the urine may be dark-coloured and gives a brownish-red colour on the addition of ferric chloride.[4] Fuchsin (hydrochloride of rosaniline $C_{20}H_{19}N_3 . HCl$) reappears partly unchanged in the urine, to which it imparts a reddish tinge. It is detected by making the urine alkaline with ammonia and shaking with an equal volume of ether: the latter extracts the colouring matter and into the solution thus obtained a thread of white wool is dipped and allowed to dry spontaneously. If fuchsin is present the wool is stained red. Salicylic acid (ortho-oxybenzoic acid, $OH . C_6H_4 . COOH$) is excreted partly in an unaltered form, partly as salicyluric acid, $OH . C_6H_4 . CONH . CH_2 . COOH$. These may be detected by the intense violet colour they yield on the addition of ferric chloride. Finally after the absorption of carbolic acid (phenol) and many other aromatic compounds such as pyrocatechin, hydrochinon, &c., the urine turns greenish-brown and finally dark-brown on exposure to air.

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[1] Otto, Pflüger's *Arch.* Bd. XXXIII. (1884), S. 613. Mester, *Zt. f. physiol. Chem.* Bd. XII. (1888), S. 130.

[2] For further literature of these red pigments see Mester, *loc. cit.* S. 143. Also *Berl. klin. Wochensch.* 1889, Sn. 5, 202, 490, 520, 953; 1890, S. 585. *Centralb. f. klin. Med.* 1889, S. 505. Stokvis (Dutch), Abst. in Maly's *Bericht.* 1889, S. 462.

RETINAL PIGMENTS.[5]

The pigments which have to be considered under this heading are numerous. There is in the first place the extremely stable dark-brown colouring-matter of the retinal epithelium, belonging to that general class of pigments known as melanins (see p. 256) and called in this case *fuscin*. In addition to this the retinal epithelium of some animals contains a not inconsiderable amount of fat globules whose yellow colour is due to *lipochrin*, a pigment closely allied to that of other fats of the body and known under the generic name of lipochromes or luteins. Passing from the epithelium to the retina proper we find in the outer end of the inner limb of the cones highly coloured fat globules from which three distinct pigments known as chromophanes, also belonging to the general class of lipochromes, may be obtained; to these the names *rhodophane, chlorophane,* and *xanthophane* have been given in correspondence with their respective red, green, and yellow colours. In addition to the above the outer limbs of the rods (not the inner limbs or either the inner or outer limbs of the cones) after the retina has been shielded for some time from the action of light, are found to present a distinct reddish-purple colour which is very marked when the retina is examined as a whole. This colour[1] is due to an exceedingly unstable[2] pigment called by Kühne 'visual-purple' or rhodopsin. The stability of the above pigments other than visual-purple is merely relative not absolute, since they are all sooner or later destroyed (bleached) by sufficiently prolonged exposure to light. The possibilities hereby suggested of a photochemical explanation of retinal excitation have however as yet thrown no real light on the nature of the process. It may be that the impulses result from the changes which these pigments undergo, and it is possible that the coloured globules of the cones play a part in the whole process not merely by the instability of their colours but also by acting as coloured though transparent screens, and thus at the same time determining the advent to the photochemical apparatus of rays of certain wave-length only. Such speculations are interesting but for the present devoid of any decisive experimental support (§ 773).

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[1] For discrimination of these see Munk, Virchow's *Arch.* Bd. LXXII. (1878), S. 136.

[2] Quincke, *Arch. f. exp. Path. u. Pharm.* Bd. XVII. (1883), S. 273.

[3] Baumann, *Zt. f. physiol. Chem.* Bd. VI. (1882), S. 193.

[4] Umbach, *Arch. f. exp. Path. u. Pharm.* Bd. XXI. (1886), S. 161.

[5] The following account of these pigments is based upon Kühne's article in Hermann's *Hdbch. d. Physiol.* Bd. III. Thl. 1. 1879, and on the original papers in Kühne's *Untersuch. a. d. physiol. Inst. zu Heidelberg*, 1878—1882, in which the literature is fully quoted.

1. **Fuscin** (Retinal melanin).[3]

This pigment is found as minute granules imbedded in the cell-substance and processes of the retinal epithelium (see § 746). These granules may be either irregular, as they always are in the choroid, or may, especially as in birds, possess an elongated form with sharply pointed ends distinctly suggestive of a crystalline structure. It is obtained by extracting the tissues with boiling alcohol, ether, and water, and then digesting for some time with trypsin. The residue is freed from nucleins by dissolving the latter in caustic alkalis, and from neurokeratin (p. 87) by decantation and straining through fine gauze. The pigment when freshly prepared is practically insoluble in all ordinary reagents, but is partially dissolved if boiled for some time with strong caustic alkalis or sulphuric acid. By prolonged treatment with dilute nitric acid it becomes soluble in alkalis, yielding yellow solutions. It becomes similarly soluble by prolonged exposure to light with free access of air (oxygen) and may be again precipitated from these solutions by the addition of an acid. It is remarkable that notwithstanding its extreme insolubility and resistance to the action of most reagents fuscin is gradually bleached by exposure to light, a result due to some oxidational change since it only occurs in presence of oxygen. The product to which the above description refers contains much nitrogen, and leaves on incineration a slight ash-residue containing traces of iron.

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[1] First observed in the retina of vertebrates by H. Müller (1851), and extended by Leydig in 1857.

[2] The instability on exposure to light was first described by Boll, 1876.

[3] The pigments of the retinal epithelium and choroid are apparently identical.

Later investigations of the pigment (from the choroid and iris) confirm the above statements of its insolubility in most reagents, and further show that it contains neither sulphur nor iron. The black pigment from hairs is stated to contain less nitrogen and a not inconsiderable amount of sulphur but no iron, and to be readily soluble in alkalis.[1] When the several substances described under the general term melanins are compared each with the other it is found that they are by no means identical, but in the absence of any guarantee of the purity of each product or of the absence of change during its preparation, all specific statements of differences must be received with caution. Possibly they are all closely allied and probably in some cases, as in the melanæmia of the malarial fever[2] or the melanuria (and melanotic pigmentation) accompanying certain kinds of tumours (p. 256), they are derived from the colouring-matter of the blood. The divergence in views as to their derivation from hæmoglobin has apparently turned in many cases on the presence or absence of iron in the pigments under examination. Some of the melanins may contain iron, some none, but whether they do or do not is not a decisive test of their derivation. If they do it makes the connection more probable, if they do not they may still take their origin from blood-pigments, as in the case of the highly coloured but iron-free hæmatoporphyrin.

2. **Lipochrin.**

The fat globules in the retinal epithelium from which this pigment is obtained are more especially abundant in the frog. It is soluble in chloroform, ether, benzol, carbon bisulphide, &c. When dissolved in ether it gives two absorption bands between *F* and *G*; in carbon bisulphide two bands, one each side of *F*.[3] The pigment of the body-fat of frogs gives similar absorption spectra when dissolved in the same solvents. Solutions of lipochrin are slowly bleached by exposure to a strong light. The pigment is probably closely allied to the yellow colouring-matter of many other animal fats. (See below sublutein.)

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[1] Sieber, *Arch. f. exp. Path. u. Pharm.* Bd. xx. (1886), S. 362.

[2] For references see Gamgee, *Physiol. Chem.* Vol. I. (1880), p. 162.

[3] See Kühne and Ayres, *Jl. of Physiol.* Vol. I. (1878), p. 109.

3. **Chromophanes.**[1]

These are, as stated above, the colouring-substances of the fat-globules which occur between the outer and inner limbs of the retinal cones. They are prepared, as yet chiefly from the eyes of birds, as follows. The retinas are dehydrated with alcohol and extracted with ether. The ethereal solution of the fats is then evaporated to dryness, the residue dissolved in hot alcohol and saponified with caustic soda. The hard coloured soaps thus obtained are then extracted in succession with petroleum ether (see note p. 156), ether, and benzol: of these solvents the first dissolves out the yellowish-green chlorophane, the second the yellow xanthophane, and the third the red-coloured rhodophane.

(i) *Chlorophane.* Soluble in petroleum ether, ether, carbon bisulphide, and in alcohol. When dissolved in the first two of these solvents it shows two absorption bands between *F* and *G*; in solution in the latter, the two bands lie one each side of *F*.

(ii) *Xanthophane.* Soluble in ether, carbon bisulphide, and in alcohol. In ethereal solution it shows only one absorption band, near *F*, towards the blue end of the spectrum. In carbon bisulphide it shows similarly one band near, and to the blue side of, *b*. It is thus distinguished from the yellow pigment (lipochrin) of the retinal epithelium previously described.

(iii) *Rhodophane.* Soluble in turpentine, benzol, and in alcohol. In benzolic solution it shows one band close to, but on the red side of, *F*; in solution in turpentine the band is similarly near, but now on the blue side of, *F*.

Solutions of the chromophanes are slowly bleached by the action of light, — chlorophane losing its colour fairly rapidly, xanthophane more slowly, and rhodophane only after prolonged exposure. In the less pure form in which the chromophanes were first obtained by Kühne, they gave the reactions which characterise the lipochromes or lutein, viz.: (i) A transient violet, followed by a bright blue, when treated with *concentrated* sulphuric acid. (ii) A transient bluish-green under the influence of strong (yellow) nitric acid. (iii) An initial green colour, passing into bluish-green, by the action of a dilute (·25 p. c.) solution of iodine in dilute (·5 p. c.) iodide of potassium.[2] In the purer form in which they were subsequently prepared, Kühne found that they all three gave the first of the above reactions, while none of them were coloured by the iodine solution, and in the case of rhodophane the second reaction with nitric acid was scarcely marked.

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[1] Kühne and Ayres, *loc. cit.* and *ibid.* p. 189.

[2] See Capranica, *Arch. f. Physiol.* 1877, S. 283. For a conclusive reply to the views as to the identity of these fatty pigments with lutein, put forward in this paper, see Kühne, *Unters. a. d. physiol. Instit. Heidelb.* Bd. IV. (1882), S. 169.

4. **Visual-purple** (*Rhodopsin*).

This extremely unstable pigment may be stated to occur generally (some few exceptions have been observed) in the retinæ of all vertebrates. It does not appear as yet to have been found in the eye of invertebrates.[1] It is confined entirely to the outer limbs of the rods, but while occurring in the majority of the rods it is not found in all of them; thus, it is absent in those situated in the immediate neighbourhood of the *ora serrata*, and (in man at least) it is wanting in the scantily disposed rods in the immediate neighbourhood of the fovea centralis. It is entirely absent from the cones, and hence is not found either in the fovea centralis of the human retina, or in the rod-free retina of reptiles.

Preparation in solution. The most suitable material is afforded by the retinæ of frogs which have been kept in the dark for two or three hours; since in these animals not only is the visual-purple very marked and somewhat persistent under the action of light, but further, the retina can be separated from the adjacent epithelium with great ease and is free from blood. The necessary operation for the removal of the retinæ, as also all subsequent manipulations, must be carried on in a feeble light from a sodium flame to avoid bleaching. The retinæ (20—30 suffice) are then extracted for an hour in the dark with about 1 c.c. of a freshly prepared 2—5 p. c. solution of bile salts from ox-bile, which is finally filtered. If brought into daylight and examined, the solution is seen to possess a brilliant pinkish-purple colour, which rapidly becomes red, yellow, and finally colourless, under the action of light. A similar initial colour is observed in the retina *in situ*, followed by the same change of colour when exposed to light, the yellow being regarded as due to a 'visual-yellow' (xanthopsin) and perhaps the final colourless stage, since it admits of regeneration in the dark into visual-purple if the retina is fresh and in contact with its epithelium (see § 773), may be spoken of as a 'visual-white' (leukopsin).

Spectroscopic properties. Neither visual-purple nor visual-yellow gives any distinct absorption band; there is a general absorption of the central parts of the spectrum easily seen between *E* and *G* in the case of visual-purple, which changes into a general absorption of the violet end of the spectrum from *F* onwards as the purple changes into yellow and finally disappears altogether.

Action of light. White light, as also that from an electric lamp or magnesium flame, bleaches visual-purple with extreme rapidity, dependently upon the intensity of the illumination: direct sunlight destroys the colour almost instantaneously.

---

[1] The red colour of the retina of Cephalopods, first described by Krohn in 1839, is due to other pigments which are very resistent to the action of light.

When monochromatic light (of the spectrum) is used, it is found that the yellowish-green, *i. e.* the region most strongly absorbed by the pigment, is most active, followed seriatim by green, blue, greenish-yellow, yellow, violet, orange, and red: the ultra-red rays have no such bleaching power. At low temperatures the effect of light is less, increases with rise of temperature, and at 75° the colour is destroyed even without exposure to light.

Action of reagents. The colour is at once destroyed by the action of caustic alkalis, most acids, alcohols, chloroform, and ether: it is on the other hand persistent in presence of ammonia, solutions of ordinary alum, of sodium chloride, carbonates of the alkalis, and a large number of other salts. One of the most important factors in determining the bleaching of visual-purple by either light or heat is the presence or absence of water. If the entire retina be dried in vacuo over sulphuric acid, or if a solution of the pigment be similarly evaporated to dryness, the visual purple is comparatively resistent to the action of light, although it is bleached by a sufficiently prolonged exposure.

LIPOCHROMES OR LUTEINS.

After the rupture of the ovarian follicle which accompanies the discharge of an ovum, the cavity of the follicle becomes filled with a mass of cells, traversed by ingrowths of connective tissue from the neighboring stroma, and frequently contains blood resulting from hæmorrhage at the time of rupture (§ 934). This is followed, most strikingly if impregnation of the discharged ovum takes place, by a fatty degeneration of the contained cells, resulting in the formation of a bright pigmented mass of a brilliant yellow or orange colour, while at the same time the colouring-matter of the blood may be converted into that crystalline substance already described under the name hæmatoidin (p. 239) as being identical with bilirubin. The structure which results from the above changes is known as a 'corpus luteum.' The earlier (1868) examination of coloured extracts of these corpora lutea led to erroneous statements of the identity of the pigment obtained from them with hæmatoidin, — a view which was almost immediately contested, — while the colouring matter received the name of hæmolutein. A renewed investigation of the pigment led Thudichum[1] to characterise it as of wide-spread occurrence in the highly coloured fatty constituents as of butter, fats, egg-yolk, &c., and of some vegetable tissues, and to give it the name lutein, under which designation as a class-name these fatty pigments have usually been known. Since, however, as we have already seen in the case of the chromophanes, and as will appear subsequently in the case of the pigments of egg-yolk, and of the substance tetronerythrin, we have to deal with pigments which, while they give the reactions characteristic of the group, exhibit colours other than yellow, it is perhaps advisable now to use the term 'lipochrome' as generic, and to retain lutein as specific for certain yellow pigments only. The lipochromes are characterised by exhibiting absorption bands which, though varying somewhat in position according to the solvent employed, are usually situated towards the violet end of the spectrum. From a chemical point of view the reactions already described on p. 263 may be regarded as characteristic of the whole class.

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[1] *Centralb. f. d. med. Wiss.* 1869, S. 1.

1. Lutein.[1]

This pigment may be obtained from corpora lutea by extraction with chloroform. If the orange-coloured solution thus obtained be allowed to evaporate spontaneously, a fatty residue is left in which the lutein is found in a crystalline form, as minute either rhombic prisms or plates, which are pleochromatic (see p. 216). They are insoluble in water, but readily soluble in alcohol, ether, chloroform, and benzol. These exhibit two absorption bands, one inclosing *F*, the other about half way between *F* and *G*.

If egg-yolk be extracted with a little alcohol and much ether, the solution shows two bands similar to those already described for lipochrin or frog's fat (p. 262), while sometimes a third faint band near *G* may be seen, especially if the residue from the ethereal extract be dissolved in carbon bisulphide and examined. If the residues from the ethereal extracts of egg-yolk and corpora lutea be saponified and extracted with carbon bisulphide, the solutions yield identical absorption spectra.[2]

Maly,[3] operating on the bright red eggs of a sea-spider (Maja Squinado) considered that lutein (assuming its identity in this case with that from ordinary egg-yolk) consists of two pigments, vitellolutein (yellow) and vitellorubin (red). For further details see the original paper. Lutein is more or less rapidly bleached by the action of light.

2. Serum lutein.

The serum from the blood of almost all animals is usually of a more or less yellow colour; it is specially marked in the case of the horse and ox, is also marked in the case of sheep and man, and is but slightly present under normal conditions in the serum of the dog, rabbit, or cat. The colour has by different observers been ascribed to different pigments. In some cases it may be due, at least partly, to the presence of bile-pigments or their derivatives,[1] these being much increased in certain diseases, such as jaundice. But in addition to these it appears that the colour of all pigmented serums is due to a specific pigment, which, while it may differ (?) slightly as obtained from the blood of different animals, belongs in each case to the general class of substances known as lipochromes. This view was originally put forward by Thudichum,[2] who ascribed the colour to the pigment lutein, which has been already described. This view is probably correct, independently of the possibility that the colour may be in some cases due partly to the simultaneous presence of bile-pigments or their derivatives. Thus it is found[3] that by shaking serum with ethyl or amyl alcohol a coloured extract is obtained which contains a fatty pigment, evidently belonging to the class of lipochromes, as judged by the fact that it is soluble in alcohol, ether, chloroform, benzol, carbon bisulphide, &c., shows the two (in the case of birds only one) bands in the blue part of the spectrum, and gives the chemical reactions (p. 263) with nitric acid and sulphuric acid characteristic of these substances. It is in many cases identical with the pigment which can be extracted from the fat of the animal from whose blood the serum was obtained. Serum-lutein is bleached by the action of light.

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[1] See Capranica, *loc. cit.* on p. 263.

[2] Kühne and Ayres, *Jl. of Physiol.* Vol. I. (1878), p. 127. Gives spectra.

[3] *Monatshefte f. Chem.* Bd. II. (1881), S. 18. Gives literature to date. See recently Bein, *Ber. d. d. chem. Gesell.* Bd. XXIII. (1890), S. 421.

3. **Tetronerythrin.**

This name was first given to a substance extracted by chloroform from the red excrescences over the eyes of certain birds.[4] It was subsequently investigated by Hoppe-Seyler (from the same source), and described later as occurring in some sponges,[5] fishes,[6] and feathers.[7] More recently it has been found as a pigmentary constituent of the blood of crustacea.[8] The pigment is readily soluble in alcohol, ether, chloroform, benzol, and carbon bisulphide, is readily bleached by light, yields the chemical reactions with sulphuric acid, nitric acid, and iodine, which are characteristic of the lipochromes (see p. 265), like these shows an absorption band near *F* somewhat similar to that of xanthophane and rhodophane (p. 263), and is slowly bleached by the action of light.

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[1] Hammarsten (Swedish). See Maly's *Jahresb.* 1878, S. 129 (Bilirubin in blood-serum of horse but not of ox or man). Maly, Liebig's *Annal.* Bd. 163 (1872), S. 77 (Hydrobilirubin). MacMunn, *Proc. Roy. Soc.* Vol. XXXI. (1880), p. 231 (Choletelin).

[2] *Centralb. f. d. med. Wiss.* 1869, S. 1.

[3] Krukenberg, *Sitzb. d. Jena. Gesell. f. Med. u. Naturwiss.* 1885. Halliburton, *Jl. of Physiol.* Vol. VII. (1885), p. 324.

[4] Wurm, *Zt. f. wiss. Zool.* Bd. XXXI. (1871), S. 535.

[5] Krukenberg, *Centralb. f. d. med. Wiss.* 1879, S. 705.

[6] Krukenberg, *Vergleich.-physiol. Stud.* 1 Reihe, Abth. 4, 1881, S. 30.

[7] Krukenberg, *Ibid.* Abth. 5, S. 87. 2 Reihe, Abth. 1, S. 151. See also Merejkowski, *Compt. Rend.* T. XCIII. (1881), p. 1029. MacMunn, *Proc. Roy. Soc.* Vol. XXXV. (1883), pp. 132, 370.

[8] Halliburton, *Jl. of Physiol.* Vol. VI. (1884), p. 324.

The pigments of the animal body which have been so far dealt with admit of a certain amount of classification with reference either to the secretions or organs in which they occur, to their genetic relationships each with the other, or in some cases (lipochromes) to their probable chemical similarities. But in addition to these an extremely numerous mass of pigments has been at different times described under various names, as obtained from the brightly-coloured parts of invertebrates and of vertebrates, such as the feathers, &c. Our knowledge of them is quite incomplete and limited in most cases to statements of their solubilities and the absorption spectra which some of them yield. In most cases nothing is known of their chemical nature or their relationships (if any) to each other, and any description of them even if it were profitable, is impossible within any reasonable limits.

For details and references to the literature of the several pigments see Gamgee, *Physiological Chemistry*, Vol. I. 1880, p. 305, and particularly Krukenberg, *Vergleichend-physiol. Studien*, Heidelberg, 1881–1888 and *Vergleich. physiol. Vorträge*, Bd. I. 1886, Nr. 3.

In conclusion it must suffice to describe two pigments which do not naturally fall under any of the above groups into which these substances have been divided.

Pyocyanin.[1] Pus, which ordinarily presents a more or less bright yellow colour, is frequently greenish and sometimes blue. The blue colour is due to a pigment (pyocyanin) which is apparently formed in the pus by the action of specific organisms. It is obtained either from pus or the bandages into which it has been absorbed by extraction with dilute alcohol or with water to which a trace of ammonia has been added. The alcoholic extract is then evaporated to a small bulk and the residue extracted with chloroform, or it may be extracted at once from the aqueous solution by shaking with chloroform. It may be obtained in a crystalline form by slow evaporation of the chloroformic solutions, the crystals being readily soluble in water and alcohol, but only slightly in ether. Acids change the blue colour to red, and alkalis restore the original blue. None of the solutions show any distinct absorption bands. When kept the crystals turn greenish, due to a decomposition which takes place most readily in alkaline solutions exposed to the air and light, and results in the formation of a yellow pigment, pyoxanthose. The latter is, unlike pyocyanin, only slightly soluble in water, but readily soluble in ether, by which property the two pigments admit of being separated. Pyoxanthose is crystalline, soluble in alcohol and chloroform, is coloured red by acids and violet by alkalis. Since pyoxanthose appears to be a product of the decomposition of pyocyanin, both pigments may occur simultaneously in pus, in which case the fluid is green. According to some more recent observations [1] pyocyanin, as judged of by its reactions with the chlorides of gold and platinum and with other alkaloidal precipitants, as also from the formation of crystalline compounds with acids, is closely related to the alkaloids.

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[1] Fordos, *Compt. Rend.* T. LI. (1860), p. 215; *Ibid.* LVI. (1863), p. 1128. Lücke, *Arch. f. klin. Chirurg.* Bd. III. (1863), S. 135. Girard, *Deutsch. Zeit. f. Chirurg.* Bd. VII. (1876), S. 389. Fitz, *Ber. d. d. chem. Gesell.* Bd. XI. (1878), Sn. 54, 1893. Kunz, *Monatsh. f. Chem.* Bd. IX. (1888), S. 361.

Sweat is also occasionally coloured blue, in some cases by indigo-blue (p. 200) as in urine, and it may be (?) by a pigment similar to pyocyanin.

Pigment of the suprarenal bodies. A suprarenal body when a section is made through it is found to consist of an outer or cortical portion, of a yellow colour, which constitutes the chief part of its structure, and an inner, medullary part of a darker colour. When the latter is acted upon by ferric chloride it assumes a dark bluish- or greenish-black colour, and if an aqueous extract of its substance (or the tissue itself) be treated with an oxidising agent it turns red (§ 498). It appears therefore that the suprarenals contain some form of chromogen or pigment-forerunner which gives rise under appropriate conditions to a pigment. According to some observers extracts of the cortex show a spectrum similar to that of the histohæmatins (p. 234) while the medulla gives one resembling hæmochromogen.[2] The pigment obtainable from the suprarenals has been investigated by Krukenberg.[3] By a method for which the original paper must be consulted, he isolated a brownish-red substance with an acid reaction, soluble in water and alcohol, whose reactions were the same as those of extracts of the suprarenals. None of the solutions showed any distinct absorption bands. The whole subject requires further investigation, which might be of interest in connection with the origin and causation of the increaesd pigmentation of the skin observed when the suprarenals are diseased.

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[1] Gessard, *Compt. Rend.* T. XCIV. (1882), p. 536.

[2] Mac Munn, *Proc. Physiol. Soc.* Dec. 1884 (*Jl. of Physiol.* Vol. v. p. xxiv).

[3] Virchow's *Arch.* Bd. CI. (1885), S. 542.

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